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The impact of processing techniques on tea volatiles

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The impact of various cultural and manufacturing techniques on volatile flavour composition was studied in order to optimize the conditions for production and retention of aroma in relation to tea quality. The Flavour Index was in the order: clonal variation—Assam > > Cambod > China Shoot maturity—Bud + 1st leaf > > > 2nd leaf > > 3rd leaf; plucking interval 7 day > 14 day; processing—green leaf > withered leaf—fermented dhool < dried tea < < tea brew; withering—soft < normal < hard. VFC Group I was in general dominated by *trans*-2-hexenal and Group II by linalool, phenylacetaldehyde and geraniol. Fresh green leaf had a high content of hexanol, hexanal, hexenol, hexenal and methyl salicylate. Upon withering, a sharp increase in Group I was noticed, the most remarkable being in hexenol. Group II also increased, but the extent was less except for linalool. During fermentation, Group I alcohols showed a sharp reduction with concomitant increases in aldehydes, especially *trans*-2-hexenal. In Group II, all compounds increased except methyl salicylate and the ionones. In the firing stage, high losses of Group I and Group II were registered. All the Group I compounds showed a decline with the progress of withering, but the opposite applied to Group II compounds, except for the alcohols. Mechanical injury during handling of leaf before cutting increased the Group I content enormously. The addition of exogenous fatty acids, mainly linoleic acid, produced substantial amounts of Group I compounds, dominated by *trans*-2-hexenal and hexanal. The inhibition of lipoxygenase totally reduced the formation of Group I volatiles.

INTRODUCTION

Tea (*Camellia sinensis* L. (O) Kuntze), being an important agronomic crop, is cultivated in more than 3 million hectares 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. 'Quality' has gained increasing importance as consumers have become more health-conscious and thus quality control has become an integral part of modern tea technology (FAO, 1989).

The widespread occurrence of flavonoids, proteins 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 (Hampton, 1992). Tea aroma, which is composed of the volatile flavour compounds (VFC) generated during tea processing, was recently demonstrated to be an important quality parameter determining the price of made tea. These VFC can be divided into two groups (Owuor *et al.*, 1990). The Group I compounds are products of lipid breakdown, mainly of the unsaturated fatty acids, mediated by the action of lipoxygenase (Mahanta *et al.*, 1993). Thus, linolenic acid forms *cis*-3-hexenal, which is partly reduced to *cis*-3-hexenol by alcohol oxidoreductase and partly isomerized to *trans*-2-hexenal by isomerase. Some of this, in turn, is reduced to *trans*-2-hexenol (Robinson and Owuor, 1992). Likewise, linoleic acid forms *n*-hexanal, which reduces to *n*-hexanol. In the same manner, oleic and palmitoleic acids produce *n*-nonanal and *n*-nonanol and *n*-heptanal and *n*-heptanol, respectively (Horita and Owuor, 1987). Similarly, 1-octen-3-one and 1-octan-3-ol are produced from linoleic acid and 1-penten-3-one, 1-penten-3-ol, *cis*-3-penten-1-ol and *cis*-3-penten-1-one are produced from linolenic acid by the action of oxidoreductases and isomerases (Ganeshan and Ramasamy, 1996). The mechanism of the formation of these compounds has been worked out and shown to occur via regio- and enantio-selective formation of 13-hydroperoxy-*cis*-9*trans*-11*cis*-15-octadecatrienoic acid in the case of

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linolenic acid (Kajiwara *et al.*, 1982) and 13-hydroperoxy-*cis*-9*trans*-11-octadecadienoic acid for linoleic acid (Hatanaka *et al.*, 1979). These products of fatty acid degradation during black tea manufacture make up over 90% of Group I, which imparts an undesirable grassy odour. However, the Group II compounds, which impart a sweet flowery aroma to black tea, are mainly derived from glycosides of terpenoid related compounds (Robinson and Owuor, 1992). They comprise terpenoids, aromatics and other non-terpenoids. They are formed by enzyme-initiated oxidative breakdown of carotenoids (Sanderson and Graham, 1973) and by hydrolysis of terpenoid glycosides (Takeo, 1981). They are also derived from amino acids and sugars through the Strecker degradation and the Maillard reaction, which are very important in the non-enzymic browning of foods (Owuor and Orchard, 1989; Nawar, 1969). All these volatile flavour compounds have been recorded in Indian clonal black teas. While the biogenic pathways of aroma formation have been thoroughly studied (Robinson and Owuor, 1992), the changes occurring in their composition at various stages of manufacture have yet to be worked out. It is known that the flavour of made tea depends on the Flavour Index (Obanta and Owuor, 1995*a*), which is the ratio of the sum of VFC Group II to that of VFC Group I, whose compositions vary depending on the processing techniques. The main objective of this study was to discover the variation in VFC formation as influenced by different cultural and processing techniques in order to describe a method for assessing flavour potential and to understand the applicability of the different biogenic mechanisms.

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. They were withered at a thickness of 20 cm with a constant air-flow of 25 cfm/kg of leaves at a hygrometric difference of 6°F maintained for 16 h. The withered leaves were subjected to crush, tear, curl (CTC) rolling in a continuous 4 cut system. The machine rolled leaves were fermented to their optimum fermentation period (TRI standard) and fired at 130°C for 30 min with hot air to obtain black tea containing 3% moisture. Only materials passing between sieves BSS 18–24 mesh number were taken for analysis.

The bud and first leaf, second leaf and third leaf of each shoot were separated out from the plucked (three and a bud) and manufactured separately to study their individual VFC contribution. The leaves were classified as hard withered, soft-withered and normal-withered by fixing the moisture content of the withered leaf to 53, 73 and 65%, respectively. Injured leaves were prepared by macerating fresh green leaves in a mortar and allowing them to brown for 2 h at room temperature. Samples with added fatty acids were prepared by adding 500 μl of linoleic acid and linolenic acid in different batches of green leaves at the third CTC cut by uniformly mixing them with the cut dhool and then continuing manufacture in the conventional manner. For the inhibition studies, appropriate volumes of NaCN solution (0.2 M) were blended with the macerated dhool.

Analysis of volatile flavour compounds

VFC were extracted by placing 100 g of black tea (500 g green leaf), collected from each of the different batches, in a separate 2-litre round-bottom flask containing 1 l deionized hot water. Dichloromethane (70 ml) was used as the extracting solvent in the simultaneous distillation and extraction (SDE) method. The separation of volatile compounds was carried out under reduced pressure (150 mm Hg, 70°C) for 60 min. The condenser of the SDE head was cooled with a mixture of water and ethylene glycol at −5°C. After the addition of 1 ml of internal standard (50 mg ethyl caproate in 500 ml 5% ethanol), the extract was dried over anhydrous sodium sulfate and concentrated to about 100 μl.

Gas chromatography

A Shimadzu GC-14A instrument, equipped with a 60 m×0.25 mm i.d. DB-Wax film thickness 0.25 μm fused-silica capillary column and a flame ionization detector (FID), was used. The oven temperature was programmed from 50 to 230°C at 2°C/min. The injector and detector temperatures were 200 and 250°C, respectively. Compounds were identified by comparision of the GC retention times with those of authentic chemicals (Sigma). A GC-17A, equipped with QP-5000 (quadrupole) mass spectrometer, was used to confirm the GC compounds. MS ion source temperature was 200°C and electron energy was 70 eV. The GC kovats index and MS fragmentation pattern of each compound were compared to those of the authentic compounds. The method used is 100% reproducible with respect to extraction and analysis. The sensitivity was as low as 0.01 ppm. A typical gas chromatogram of tea volatiles is shown in Fig. 1.

RESULTS AND DISCUSSION

Study of clonal variations helps in selecting the elite clone with desirable yield and/or quality, which can be multiplied by either tissue culture or vegetative propagation or grafting techniques. The data obtained on clonal and varietal variation in VFC is summarized in Table 1. Assam, China and Cambod are the three major genetically diverse cultivars available in South India. The data clearly show that they differ widely in their

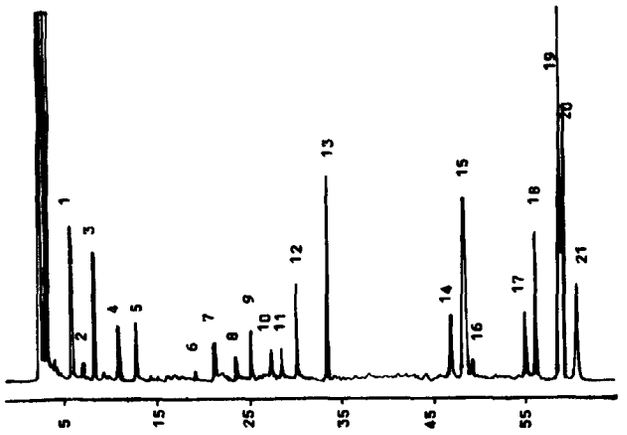

Fig. 1. Gas chromatogram of tea volatiles. The peaks numbered from 1 to 21 are *n*-hexanal, *cis*-3-hexenal, 1-penten-3-ol, *trans*-2-hexenal, pentanol, *n*-hexanol, *cis*-3-hexenol, *trans*-2-hexenol, linalool oxide I (*trans*, furanoid), linalool oxide II (*cis*, furanoid), benzaldehyde, phenylacetaldehyde, linalool, linalool oxide III (*trans*, pyranoid), methyl salicylate, linalool oxide IV (*cis*, pyranoid), geraniol, benzyl alcohol, 2-phenylethanol, α-ionone and β-ionone, respectively.

VFC composition, even though they were grown under similar conditions. Assam tea showed a higher Flavour Index and China the least with Cambod falling in between. Group I was dominated by *trans*-2-hexenal, and Group II by linalool, phenylacetaldehyde and geraniol. The high content of Group I is due to the higher proportion of unsaturated fatty acids being oxidized with higher lipoxygenase activity and content (Mahanta *et al.*, 1993). The trend observed in the Flavour Index was not found with the sum of Group I and Group II. Thus it is always the ratio of Group II to I that determines the flavour potential and not their quantity. Also, as the flavour intensity differs between individual volatiles, the Flavour Index represents only an arbitrary value (Dixon and Hammond, 1984). Each volatile has its own threshold level and only when its concentration lies beyond this does it contribute to flavour. The relative importance of a compound to flavour is related to the ratio between its concentration and its threshold concentration.

It is established that shoot maturity affects tea quality in terms of biochemical composition (Obanta and Owuor, 1995*b*). Table 2 shows the VFC variation with shoot maturity and plucking intervals. The levels of various volatiles identified were found to show significant variation with shoot maturity. The Group I compounds were found to increase with shoot maturity due to the increased lipid content. However, Group II compounds showed the opposite trend. The Flavour Index, as a consequence, declined sharply with shoot maturity. Among the Group I compounds, the most substantial increase with increased shoot maturity was that of *trans*-2-hexenal, which has a grassy and greenish aroma. Within Group II, linalool, linalool oxide and 2-phenylethanol showed the largest decline with shoot maturity. Thus the 'potential' of the different parts of shoots for flavour production has been established.

Table 1. Clonal variation in volatile flavour components of CTC black teas[a]

VFC	PN	KI[b]	UPASI-3 (Assam)	UPASI-9 (China)	UPASI-17 (Cambod)
Group I					
1-Penten-3-ol	3	1160	0.11	0.05	0.06
n-Hexanal	1	1075	0.17	0.26	0.20
n-Hexanol	6	1345	0.03	0.04	0.03
cis-3-Hexenal	2	1135	0.22	0.36	0.28
trans-2-Hexenal	4	1195	1.59	3.11	1.44
cis-3-Hexenol	7	1385	0.09	0.07	0.07
trans-2-Hexenol	8	1405	0.11	0.11	0.10
Pentanol	5	1230	0.07	0.05	0.08
Group II					
Linalool	13	1545	2.03	0.92	0.55
Linalool oxides	9,10, 14,16	1430/1460/ 1725/1750	0.26	0.13	0.09
Methyl salicylate	15	1745	0.67	0.49	0.23
Phenylacetaldehyde	12	1515	1.16	1.31	1.28
Geraniol	17	1840	1.22	1.07	0.59
Benzyl alcohol	18	1855	0.18	0.15	0.10
2-Phenylethanol	19	1875	0.73	0.43	0.48
Benzaldehyde	11	1495	0.07	0.07	0.08
α-Ionone	20	1895	0.56	0.36	0.12
β-Ionone	21	1940	0.43	0.27	0.19
Sum of Group I			2.39	4.05	2.26
Sum of Group II			7.31	5.20	3.71
Flavour Index (II/I)			3.06	1.28	1.64

[a]As ratio of peak area to that of internal standard.
[b]KI—kovats index on DB-Wax.
PN—peak number in GC.

Table 2. Impact of shoot maturity and plucking interval on VFC[a]

VFC	Shoot maturity			Plucking interval	
	Bud + 1st leaf	2nd leaf	3rd leaf	7 days	14 days
Group I					
1-Penten-3-ol	0.03	0.14	0.21	0.17	0.20
n-Hexanal	0.06	0.19	0.26	0.22	0.55
n-Hexanol	0.00	0.05	0.07	0.02	0.05
cis-3-Hexenal	0.04	0.22	0.31	0.21	0.27
trans-2-Hexenal	1.05	1.76	2.51	1.78	2.01
cis-3-Hexenol	0.01	0.11	0.17	0.03	0.10
trans-2-Hexenol	0.02	0.13	0.21	0.06	0.09
Pentanol	0.00	0.09	0.14	0.02	0.02
Group II					
Linalool	2.44	0.62	0.21	1.93	1.40
Linalool oxides	0.63	0.09	0.02	0.43	0.15
Methyl salicylate	0.75	0.38	0.17	0.66	0.51
Phenylacetaldehyde	1.76	1.17	0.67	1.72	1.78
Geraniol	1.38	0.95	0.81	1.11	0.98
Benzyl alcohol	0.21	0.11	0.07	0.12	0.14
2-Phenylethanol	0.93	0.27	0.09	0.71	0.69
Benzaldehyde	0.19	0.11	0.05	0.12	0.22
α-Ionone	0.62	0.36	0.19	0.53	0.45
β-Ionone	0.56	0.32	0.17	0.47	0.49
Sum of Group I	1.21	2.69	3.88	2.51	3.29
Sum of Group II	9.47	4.38	2.45	7.80	6.81
Flavour Index (II/I)	7.83	1.63	0.63	3.11	2.07

[a]As ratio of peak area to that of internal standard.

The composition of VFC is also affected by the plucking intervals and hence influences the quality, which has great impact on the commercial valuation. Not all differences in the individual compounds are significant as assessed on the basis of absolute differences. Among the lipid-degradation products, *n*-hexanal and *trans*-2-hexenal appear to have the most significant increases when the plucking interval is increased. Linalool and its oxides show the most marked differences with the increase in the plucking interval. While linalool, its oxides, methyl salicylate and geraniol declined, benzaldehyde and phenylacetaldehyde showed an enhancement with increased plucking intervals. The Flavour Index fell substantially as the plucking interval was increased. In South India, it has been the practice to pluck three leaves and a bud at 7–10 day intervals, assuming that this stage represents the best compromise between the level of the various chemical constituents necessary for a good cup of tea and profitability.

Data obtained on the changes in the production of volatiles during different stages of manufacture are presented in Table 3. The fresh green leaf itself contains a high amount of volatiles. However, the amount of Group I compounds was found to be very much higher than that of Group II. Group I was dominated by *n*-hexanol, *n*-hexanal, *cis*-3-hexenal, *trans*-2-hexenal and *cis*-3-hexenol. In Group II, only methyl salicylate and phenyl acetaldehyde were present in comparable quantities. However, the process of withering brings about great changes in VFC. In general, Group I was enhanced with great increases in *n*-hexanol, *cis*-3-hexenol and *trans*-2-hexenal. All components of Group II except benzyl alcohol and benzaldehyde were also enhanced, linalool giving the greatest increase. This is of interest because it influences the aroma of black tea to a considerable extent. Altogether different trends were noticed during fermentation (oxidation). Whilst all the aldehydes of Group I were enhanced, the alcohols, except *trans*-2-hexenol, declined upon fermentation. Linalool oxides increased during fermentation, along with linalool, phenylacetaldehyde and geraniol. A sharp decline was observed for methyl salicylate and the ionones were also lowered. The total amount of volatiles present in the fired tea was comparable to that in green tea, indicating that most of the compounds formed in fermentation had volatilized during the drying process. While all the Group I compounds, dominated by *trans*-2-hexenal, showed a major decline, Group II compounds behaved differently. Benzaldehyde and the ionones increased and the decline in the other Group II compounds was not as severe as that of Group I. Finally, in the brew, only a small quantity of volatiles was found. Aldehydes and ketones were almost nil and there was some retention of linalool and its oxides, but 2-phenylethanol and benzyl alcohol actually increased.

A look at the sum of Group I and Group II volatiles upon processing shows that the former increased with the progress of manufacture, but declined on drying. This is due to the high temperature used, at which the volatiles escape very easily. Group II volatiles showed a similar trend, but decreased only a little on drying. However, their ratio, the Flavour Index, declined upon

Table 3. Changes in VFC during CTC manufacturing[a]

VFC	Green leaf	Withered leaf	Fermented dhool	Dried tea	Tea brew
Group I					
1-Penten-3-ol	0.27	0.39	0.38	0.09	0.04
n-Hexanal	1.09	1.21	1.33	0.29	0.05
n-Hexanol	1.97	6.03	3.11	0.08	0.02
cis-3-Hexenal	0.90	1.47	2.36	0.41	0.09
trans-2-Hexenal	1.73	3.49	7.26	3.02	0.16
cis-3-Hexenol	1.27	4.32	2.87	0.11	0.04
trans-2-Hexenol	0.21	0.47	1.31	0.15	0.09
Pentanol	0.19	0.25	0.08	0.08	0.01
Group II					
Linalool	0.14	0.86	1.21	0.90	0.31
Linalool oxides	0.02	0.04	0.19	0.15	0.13
Methyl salicylate	1.37	1.57	0.58	0.51	0.22
Phenylacetaldehyde	0.66	0.84	1.33	1.29	0.09
Geraniol	0.41	0.77	1.21	1.07	0.53
Benzyl alcohol	0.39	0.18	0.15	0.13	0.27
2-Phenylethanol	0.51	0.53	0.57	0.44	0.94
Benzaldehyde	0.21	0.09	0.07	0.09	0.03
α-Ionone	0.15	0.36	0.27	0.39	0.02
β-Ionone	0.09	0.27	0.22	0.31	0.01
Sum of Group I	7.63	17.63	18.70	4.23	0.50
Sum of Group II	3.95	5.51	5.80	5.28	2.55
Flavour Index (II/I)	0.52	0.31	0.31	1.25	5.10

[a]As ratio of peak area to that of internal standard.

withering and stayed low during fermentation, but increased greatly on drying and again subsequently on brewing.

Empirical quality control, with the object of improving flavour and aroma characteristics in black tea, consists of ensuring that only properly withered leaves are used. The withering experiment was conducted by maintaining hygrometric differences of 6, 8 and 10°F 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 in total and individual volatile constituents under different degrees of withering are given in Table 4. Both quantitative and qualitative changes in volatile content occur during withering. The results show that the soft-withered leaf contains a similar amount of volatiles as the hard-withered ones, but the ratio between Group I and II compounds is the least favourable. As wither progresses, Group I and Group II compounds decrease and increase, respectively, except for the alcohols of Group II, which decrease. Thus, the Flavour Index increases progressively with the degree of wither. However, there must be a limit to this trend, as biochemical reactions require a minimum quantity of water for initiation. Indeed, the nature and degree of withering may be a way of controlling relative enzyme activities, such as hydrolytic and oxidative ones.

Experience has shown that the quality of tea is improved by proper handling of the green leaf and any mechanical injury to the green leaf before the cutting stage causes made-tea quality to deteriorate. Table 5 shows the changes in VFC on mechanical injury of green leaf. All the volatiles detected showed enhancement upon mechanical injury. However, the quantitative differences varied between the individual volatiles. The largest increases were of lipid degradation products, such as *n*-hexanal, *cis*-3-hexenal and *trans*-2-hexenal. Linalool, its oxides, phenylacetaldehyde, 2-phenylethanol and benzaldehyde, which were totally absent from the intact leaves, appeared in the injured green leaves. In order to study the role of oxygen and oxidation on volatile production in the injured leaves, the same experiment was conducted in a nitrogen atmosphere. Here, a large decrease was noticed in *trans*-2-hexenal coupled with slight increases in all the Group I alcohols. Overall, the sum of Group I compounds declined. On the other hand, methyl salicylate increased and so did the linalool oxides. All other Group II compounds showed non-significant changes. In consequence, the Flavour Index value was enhanced in the presence of nitrogen instead of air.

The enzymic formation of volatiles from unsaturated fatty acids has been demonstrated in many lipid-containing food products. The present study shows the effects of adding linoleic and linolenic acids on the VFC (Table 6). Addition of linoleic acid led to a 10-fold concentration of *n*-hexanal, with a much smaller decline in *trans*-2-hexenal. The other Group I compounds remained virtually unaffected. Addition of linolenic acid gave a marked enhancement in *trans*-2-hexenal with only minor changes in the other lipid-degradation

Table 4. Effect of different degree of withering on VFC of made tea[a]

VFC	Soft withered	Normal withered	Hard withered
Group I			
1-Penten-3-ol	0.12	0.10	0.07
n-Hexanal	0.18	0.15	0.14
n-Hexanol	0.05	0.04	0.04
cis-3-Hexenal	0.27	0.24	0.21
trans-2-Hexenal	1.77	1.63	1.49
cis-3-Hexenol	0.12	0.11	0.09
trans-2-Hexenol	0.15	0.13	0.13
Pentanol	0.09	0.07	0.05
Group II			
Linalool	1.88	1.97	2.18
Linalool oxides	0.22	0.28	0.39
Methyl salicylate	0.67	0.71	0.86
Phenylacetaldehyde	1.04	1.19	1.21
Geraniol	1.46	1.25	1.27
Benzyl alcohol	0.26	0.17	0.17
2-Phenylethanol	0.79	0.73	0.76
Benzaldehyde	0.05	0.06	0.07
α-Ionone	0.44	0.58	0.65
β-Ionone	0.46	0.51	0.63
Sum of Group I	2.75	2.47	2.22
Sum of Group II	7.27	7.45	8.19
Flavour Index (II/I)	2.64	3.02	3.69

[a]As ratio of peak area to that of internal standard.

Table 5. Effect of mechanical injury in green leaf on VFC of made tea[a]

VFC	Intact green leaf	Injured green leaf (in air)	Injured green leaf (in nitrogen)
Group I			
1-Penten-3-ol	0.14	0.28	0.29
n-Hexanal	0.81	2.14	2.12
n-Hexanol	0.08	0.19	0.24
cis-3-Hexenal	1.06	2.91	2.89
trans-2-Hexenal	1.22	2.79	1.36
cis-3-Hexenol	0.21	0.33	0.39
trans-2-Hexenol	0.19	0.30	0.31
Pentanol	0.09	0.17	0.19
Group II			
Linalool	0.00	0.25	0.24
Linalool oxides	0.00	0.05	0.08
Methyl salicylate	0.09	0.17	0.39
Phenylacetaldehyde	0.00	0.73	0.71
Geraniol	0.26	0.52	0.54
Benzyl alcohol	0.04	0.08	0.09
2-Phenylethanol	0.00	0.38	0.40
Benzaldehyde	0.00	0.02	0.04
Ionone	0.07	0.09	0.11
Ionone	0.06	0.11	0.12
Sum of Group I	3.80	9.11	7.79
Sum of Group II	0.52	2.40	2.72
Flavour Index (II/I)	0.14	0.26	0.35

[a]As ratio of peak area to that of internal standard.

Table 6. Effect of endogenous addition of fatty acids on VFC[a]

VFC	Control	Linoleic acid added	Linolenic acid added
Group I			
1-Penten-3-ol	0.09	0.08	0.13
n-Hexanal	0.19	1.87	0.21
n-Hexanol	0.03	0.07	0.02
cis-3-Hexenal	0.36	0.34	0.39
trans-2-Hexenol	1.57	1.19	2.11
cis-3-Hexenol	0.04	0.02	0.05
trans-2-Hexenol	0.03	0.01	0.03
Pentanol	0.03	0.02	0.05
Group II			
Linalool	0.56	0.57	0.56
Linalool oxides	0.14	0.13	0.13
Methyl salicylate	0.19	0.19	0.18
Phenylacetaldehyde	1.22	1.20	1.22
Geraniol	0.63	0.60	0.62
Benzyl alcohol	0.14	0.13	0.14
2-Phenylethanol	0.53	0.51	0.50
Benzaldehyde	0.08	0.08	0.09
α-Ionone	0.12	0.15	0.14
β-Ionone	0.19	0.18	0.22
Sum of Group I	2.34	3.60	2.99
Sum of Group II	3.80	3.74	3.80
Flavour Index (II/I)	1.62	1.04	1.27

[a]As ratio of peak area to that of internal standard.

Table 7. Effect of lipoxygenase inhibition on major volatiles of black tea[a]

VFC	Control	Inhibited
Group I		
1-Penten-3-ol	0.07	0.01
n-Hexanal	0.18	0.03
n-Hexanol	0.01	0.00
cis-3-Hexenal	0.31	0.06
trans-2-Hexenal	1.51	0.32
cis-3-Hexenol	0.05	0.01
trans-2-Hexenol	0.07	0.01
Pentanol	0.09	0.02
Group II		
Linalool	0.58	0.57
Linalool oxides	0.11	0.11
Methyl salicylate	0.20	0.18
Phenylacetaldeyde	1.26	1.24
Geraniol	0.62	0.63
Benzyl alcohol	0.12	0.12
2-Phenylethanol	0.50	0.52
Benzaldehyde	0.08	0.08
α-Ionone	0.10	0.11
β-Ionone	0.21	0.23
Sum of Group I	2.29	0.46
Sum of Group II	3.78	3.79
Flavour Index (II/I)	1.65	8.24

[a]As ratio of peak area to that of internal standard.

products. As expected, there was no change in the Group II volatiles upon addition of either of the two fatty acids. The Flavour Index declined upon addition of fatty acids, linoleic acid having the greatest effect. The high amounts of *trans*-2-hexenal indicate a rapid enzymic isomerization of *cis*-3-hexenal to the *trans*-2-isomer.

As lipoxygenases lead to the formation of undesirable aldehydes and alcohols, which cause a grassy odour in made tea (Ganeshan and Ramasamy, 1996), the effect of lipoxygenase activity on the volatile production was studied (Table 7). Normally, tea leaves are dried at 130°C for 30 min in the drying process, which is the last stage in tea manufacture. However, some enzyme activities survive in the made black tea. Thus, it requires greater heating to inactivate the lipoxygenases. Cyanide ion has been used traditionally to differentiate haeme-catalysed from lipoxygenase-catalysed oxidation by inhibiting the latter. To evaluate its effect on the formation of volatiles, cyanide was added to cut dhool at a final concentration of 20 mM. A substantial decline in Group I compounds occurred with practically no change in the Group II compounds. Thus, it led to a sharp enhancement in the Flavour Index. Practically no Group I volatiles were produced at 100 mM cyanide concentration.

The possible pathways for forming aldehydes and alcohols from unsaturated fatty acids in tea leaf have been presented elsewhere (Robinson and Owuor, 1992). While the straight-chain alcohols and aldehydes are formed from lipid, the terpenoids are obtained by hydrolysis of their glycosides. The aromatic aldehydes are formed from amino acids, their reduction giving rise to the corresponding alcohols. Ionones are obtained by carotenoid degradation.

The positional specificity of tea leaf lipoxygenase is not known. In the presence of oxygen, linoleic acid is oxidized to hydroperoxides. *n*-Hexanal can be formed by the cleavage of 13-hydroperoxylinoleic acid by lyases. *n*-Hexanal added to cut tea dhool homogenates was reduced to *n*-hexanol in significant amounts, indicating rapid reduction by alcohol oxidoreductase. Linolenic acid can be oxidized to 9- and 13-hydroperoxides. *cis*-3-Hexenal can be formed by cleavage of the 13-hydroperoxide. We observed that homogenates of tea leaves rapidly isomerize *cis*-3-hexenal to *trans*-2-hexenal. These two aldehydes when added to cut dhool gave an increase in the corresponding alcohols, indicating reduction by alcohol oxidoreductase. Similarly, 1-penten-3-one added to cut dhool produced 1-penten-3-ol. 1-Penten-3-one could be formed by enzymic isomerization of 16-hydroperoxide.

The present investigation has very clearly established that the VFC content and composition can be greatly altered by different processing techniques. It also provides ideas on how to develop, control and preserve the flavour of black tea. In addition, monitoring the changes in VFC constitutes an appropriate quality control method for developing and controlling optimum conditions for black tea manufacture. Further studies on tea leaf flavour enzymes, to determine positional specificity of the lipoxygenase and the specificities of hydroperoxide isomerases, lyases and alcohol oxidoreductases, are in progress.

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