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The effect of packaging conditions and storage time on the volatile composition of Assam black tea leaf

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Black tea leaf, vacuum packed in Assam and subsequently repacked in the UK in standard air packs, under vacuum or nitrogen-Xushed, was stored for up to 48 weeks. The volatile composition of the tea was assessed at zero time, after 16 weeks and 48 weeks. Six compounds were identiWed as signiWcantly diVerent at 16 and 48 weeks when compared with the zero time sample ($p < 0{\cdot}001$). These were identiWed as hexanal, *trans*-2-octenal, *trans, cis*-2, 4-heptadienal, *trans, trans*-2, 4-heptadienal, β-cyclocitral and β-ionone, the level of these being at an increased level in the standard air pack and similar to the zero time sample in the vacuum- and nitrogen-Xushed packs. These results are consistent with oxidative deterioration of the tea during storage. Subsequent canonical variate analysis was carried out using these six volatiles. Results indicated clear discrimination between the samples, in particular between the standard air packs and the other samples. This oVers the potential of using these compounds as indicators of tea aroma quality and as a means of distinguishing between tea samples based on exposure time in air.

INTRODUCTION

One highly important characteristic of black tea is its aroma. Nearly 400 volatile compounds have been identiWed in black tea (Van Straten & Maarse, 1983) and there have been several studies into factors both pre- and post-harvest which alter the volatile composition. Pre-harvest factors such as time of harvest and fertiliser regime have been shown to aVect volatile composition (Hazarika *et al.*, 1984; Owuor *et al.*, 1991). Owuor *et al.*, (1991) demonstrated an increase in total volatile content of Kenyan black tea with the application of nitrogen fertilisers. However this was not accompanied by an increase in tea quality. Total volatile content has also been shown to increase with a more coarse plucking of the tea leaf (Mahanta *et al.*, 1988) although it is during post-harvest processing that most aroma volatile production occurs. Formation of volatiles due to oxidation of lipids starts during the withering process and continues throughout rolling, fermentation, Wring and storage (Selvendran *et al.*, 1978; Mahanta *et al.*, 1985). There is some evidence that an endogenous, heat stable, metal ion protein, rather than a true lipoxygenase-type enzyme is responsible for lipid oxidation in black tea (Coggon *et al.*, 1977). Other sources of volatiles during black tea processing are the oxidation of the carotenes (Sanderson *et al.*, 1971; Hazarika & Mahanta, 1983) and the conversion of amino acids to volatile aldehydes during fermentation (Co & Sanderson, 1970).

Once processed, black tea leaf is traditionally transported by ship in foil-lined plywood tea chests or more recently in foil-laminated paper sacks. During the several months of transport and storage the tea loses astringency and Xavour and develops undesirable characteristics (Stagg, 1974). It has also been shown that black tea consumes oxygen during this period (Roberts & Smith, 1963) which suggests that oxidative deterioration may contribute to changes in aroma quality after processing.

This study was set up to investigate the eVect of packaging regimes which actively remove oxygen, as a means of maintaining the aroma quality of freshly processed black tea leaf, and the potential of using volatile proWling to discriminate between teas after storage.

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MATERIALS AND METHODS

Tea leaf (*Camellia sinensis* (L.) O. Kuntze) was grown and processed by George Williamson (Assam) Ltd in Assam on the Dirial estate and then packed under vacuum in 10 kg foil pouches for transportation to the UK. On arrival in the UK the tea was packed by Premier Beverages into standard tea bags and then packaged into 40 teabags/pouch packs under three diV-erent atmospheres. These were as follows:

(1) standard atmosphere pack, using 30μm met-allised polypropylene pouches;
(2) vacuum pack, using 12/50 μm metallised poly-ester laminated to polyethylene pouches, sealed at 20 mbar; and
(3) nitrogen-Xushed (oxygen < 1·0%), using 12/50μm metallised polyester laminated to polyethylene pouches.

The volatile composition of the tea was analysed when the tea Wrst arrived in the UK (time zero) and after 16 and 48 weeks storage at ambient temperature.

Preparation of extracts of black tea volatiles

One litre of distilled water at 95°C was added to 20·0 g of tea leaf in a 2 litre round-bottomed Xask. This was allowed to equilibrate in a water bath at 60°C after which 1ml of 0·36mM 1-bromohexane was added as internal standard. The headspace volatiles were then swept onto a 1·5mg charcoal trap at 60°C for 30 min using a modiWed version (Springett *et al.*, 1988) of the closed loop trapping technique of Grob and Zürcher (1976). After this time, the charcoal was eluted with 3 × 10μl of carbon disulphide and the volatile extract was sealed in an amber glass vial and stored at −18°C prior to analysis by gas chromatography-mass spectrometry (GC–MS).

Triplicate determinations were carried out for each sample at zero time (when the tea was Wrst packed) and after 16 and 48 weeks storage at ambient.

GC-MS analysis

Analyses were carried out on a Hewlett Packard 5970 series mass selective detector connected to a 5890 gas chromatograph and a G1034A data system. Mass spectral comparisons were made against the Wiley library of mass spectra. GC-MS conditions were as follows:

helium carrier gas Xow rate	1ml/min
injector temperature	50°C
column temperature	50°C for 2 min, 5°C/min to 220°C held at 220°C for 5min
transfer line temperature	220°C
ionising voltage	70eV
scan range	28·5—300·0 amu
scan rate	3·16/s
solvent delay	2·50 min
injection	1μl on-column

The separations were carried out on a 25m x 0·25mm i.d. fused silica WCOT capillary column with a 0·2μm CPWAX 52CB stationary phase supplied by Chrom-pack (UK) Ltd.

Statistical analyses

Integrated peak areas were normalized against the internal standard, 1-bromohexane, to remove varitions due to the extraction procedure. Log_{10} peak area data was then taken for subsequent statistical analyses.

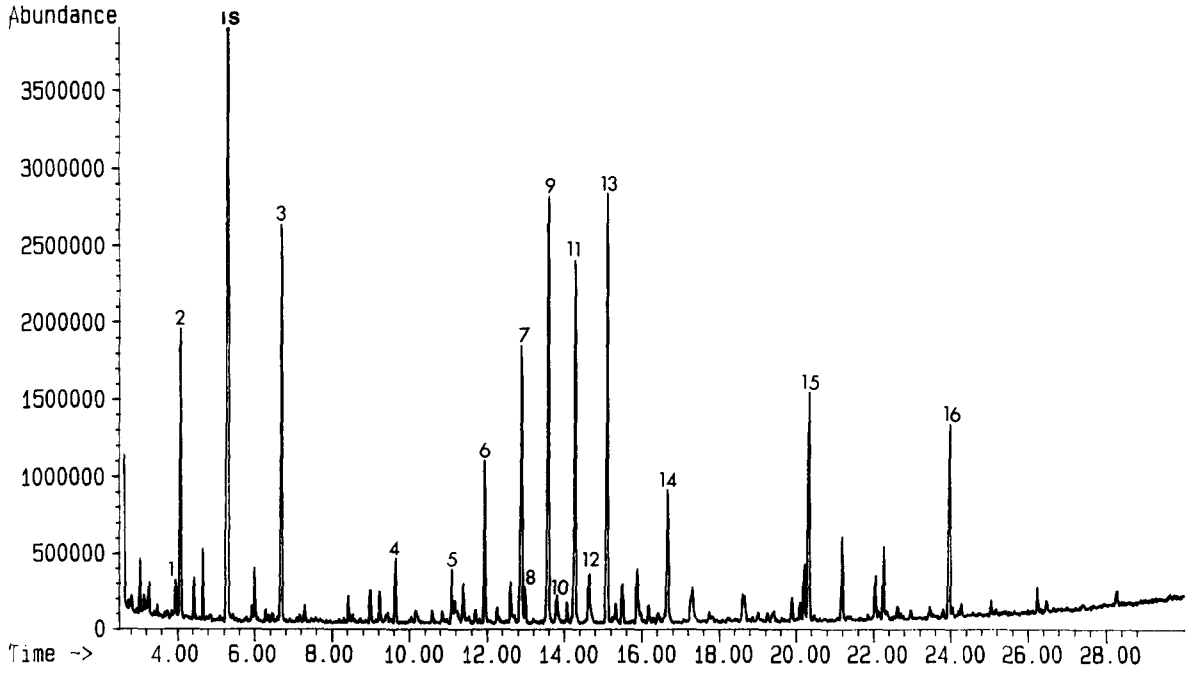

Fig. 1. Total ion chromatogram showing the 16 peaks used in the statistical analyses.

Two statistical methods were applied to the data.

(1) Analysis of variance — this was used to identify those peaks which were signiWcantly diVerent between the tea samples stored under diVerent conditions.
(2) Canonical variates — this is a multivariate technique which generates canonical variates. These are linear combinations of the measured variables; in this instance they are linear combinations of the logarithms of volatile peak areas. Canonical variates are deWned to be those which maximally discriminate or separate the diVerent experimental treatments, in this case the three packing conditions.

RESULTS AND DISCUSSION

In excess of 50 peaks were detected in the chromatograms of the black tea volatile extracts. However, this was reduced to a convenient number of 16 main peaks which were detectable consistently throughout the samples. These 16 volatiles were integrated at zero time and subsequently detected and analysed in the samples after 16 weeks storage. Figure 1 shows a typical total ion chromatogram (TIC) for a tea sample together with the 16 peaks used in the statistical treatments. Table 1 shows the data system identiWcations and peak areas for the 16 volatiles used in the statistical treatments. Results from these analyses were subjected to analysis of variance to determine those peaks which were signiWcantly diVerent between the samples. Six peaks were signiWcantly diVerent ($p < 0{\cdot}001$) when comparing the zero time sample with the 16 week samples. The results of the analyses of variance are summarised in Table 2. Where possible, the identiWcations of these six volatiles were conWrmed by retention time comparison with standard compounds. The tea samples were analysed again after 48 weeks storage and the six volatiles identiWed at 16 weeks determined. Figure 2 shows the TIC for a tea sample analysed, at zero time and after 48 weeks, packaged either in a standard air pack, under nitrogen or vacuum. Full scales on the y-axes have been adjusted to half the height of the

Table 1. Volatiles identified at 16 weeks and used in the subsequent statistical treatments

	Data system identiWcations	Peak areas at zero time and 16 weeks[a]			
		Zero time	Standard time	Nitrogen-Xushed	Vacuum-packed
1	Dimethyl disulphide	2·08	1·30	1·41	2·03
		(0·22)	(0·36)	(0·12)	(0·25)
2	Hexanal[b]	3·86	9·38	4·42	5·18
		(0·39)	(1·76)	(0·43)	(0·20)
3	*trans*-2-Hexenal[b]	25·47	25·83	30·50	36·30
		(2·91)	(5·77)	(0·53)	(2·51)
4	2,2,6-Trimethyl cyclohexanone	0·88	1·34	1·15	1·27
		(0·88)	(0·25)	(0·06)	(0·12)
5	Nonanal	3·47	3·84	2·41	2·76
		(0·34)	(1·86)	(0·16)	(0·55)
6	*trans*-2-Octenal[b]	1·12	6·09	1·58	1·59
		(0·13)	(1·15)	(0·05)	(0·10)
7	*trans,cis*-2,4-Heptadienal[b]	2·48	16·32	3·49	3·29
		(0·18)	(4·18)	(0·11)	(0·10)
8	Linalool oxide[b]	2·38	3·29	3·26	3·72
		(0·78)	(0·57)	(0·20)	(0·30)
9	*trans,trans*-2,4-Heptadienal[b]	4·24	14·37	5·48	5·18
		(0·33)	(2·84)	(0·15)	(0·66)
10	Unknown aldehyde	1·37	2·86	2·19	2·57
		(1·26)	(1·13)	(0·12)	(0·90)
11	Benzaldehyde[b]	7·59	11·43	7·75	8·84
		(0·22)	(2·02)	(0·20)	(1·18)
12	*cis*-2-Nonenal	1·35	3·16	1·95	2·01
		(0·25)	(1·04)	(0·11)	(0·10)
13	Linalool[b]	20·10	28·10	26·00	29·07
		(2·72)	(5·72)	(1·39)	(2·51)
14	β-Cyclocitral	2·81	6·68	4·68	5·23
		(0·46)	(1·04)	(0·27)	(0·31)
15	Methyl salicylate[b]	11·31	16·33	15·23	17·57
		(1·79)	(2·57)	(1·10)	(1·94)
16	β-Ionone[b]	2·09	7·43	3·32	3·77
		(0·51)	(1·70)	(0·27)	(0·11)

[a] Results shown are peak area units normalised against the internal standard and are the mean of three replicate determinations, together with the standard deviation of those means (shown in parentheses).
[b] IdentiWcation conWrmed by retention time comparison with standard compounds.

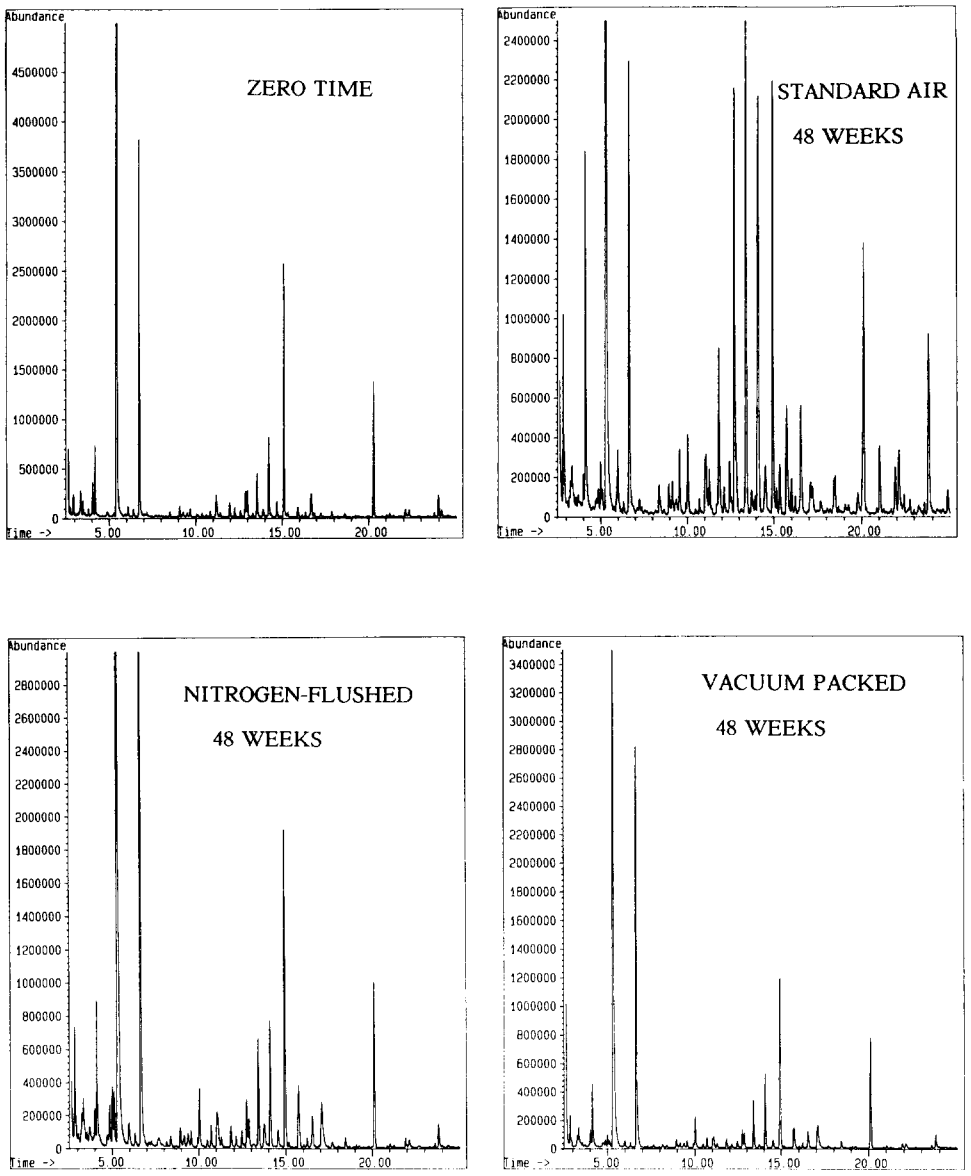

Fig. 2. Total ion chromatograms for black tea at zero time and after 48 weeks storage in standard air packs, under nitrogen and under vacuum.

internal standard compound to allow direct visual comparisons to be made between the chromatograms.

The major change in the volatile pattern of these tea samples with storage was the increase which occurred in the standard air pack compared with the zero time sample and the vacuum and nitrogen xushed sample after 16 and 48 weeks storage. Of the six peaks identiwed as signiwcantly diverent in the standard air pack, hexanal, octenal and the two isomers of heptadienal are likely to have arisen from the autoxidation of the fatty acids linolenic and linoleic (Badings, 1970) and β-cyclocitral and β-ionone from the oxidation of β-carotene. (Sanderson *et al.*, 1971). These results indicate that changes in aroma quality of black tea leaf during storage may occur due to oxidative deterioration and this change in aroma pattern is halted to some degree by the use of packaging regimes which exclude air from the pack.

These results over the potential of using these six compounds as indicators of tea aroma quality and of discriminating between teas stored for diverent times and under diverent conditions. Canonical variate analysis was applied to the six volatile peak data from the analyses at zero time, 16 and 48 weeks. Figure 3 shows a plot of canonical variate 1 against canonical variate 2; these accounted for 78 and 15% of the variation between the samples, respectively. The results from this analysis are summarised in Table 3. Clearly there is discrimination between the samples. In particular the 16 and 48 week air packs are well separated from the other samples on the x-axis and to increasing degrees with storage time. There was no discrimination between the zero time sample and the 16 week vacuum- and nitrogen-xushed samples, suggesting a stabilisation of the aroma under these storage conditions and over this time course. After 48 weeks the vacuum- and nitrogen-xushed packs showed a marked change from the zero time and 16 week packs; however, this was essentially on the y-axis. As all the compounds used in the discriminant analysis are products of oxidation, the basic

Table 2. Summary of analysis of variance at 16 weeks

Compound	16 Weeks	
	F-ratio	P^a
Hexanal	30·33	***
trans-2-Octenal	116·33	***
trans,cis-2,4-Heptadienal	107·33	***
trans,trans-2,4-Heptadienal	52·51	***
β-Cyclocitral	26·36	***
β-Ionone	25·64	***

[a] *** highly signiWcant ($P < 0·001$).

causes of these changes on the *x* and *y*-axes are likely to be the same. However, it is interesting to note that octenal is having a major inXuence in the Wrst canonical variate (*x*-axis) whilst the two isomers of heptadienal are more important in the second canonical variate (*y*-axis). This may indicate a change in the pattern of fatty acid oxidation between the standard air packs, where oxygen is in excess and the 48 week vacuum and nitrogen Xushed samples, where only traces of oxygen are available.

CONCLUSIONS

Over the time course of this study changes did occur in the volatile composition of black tea leaf. These changes were consistent with oxidation of non-volatile components and could be minimised by excluding air from the packs.

Results have also indicated the potential of using GC proWling of six aroma compounds followed by canonical variate analysis to determine tea aroma quality and to discriminate between teas stored for diVerent times and under diVerent packaging conditions.

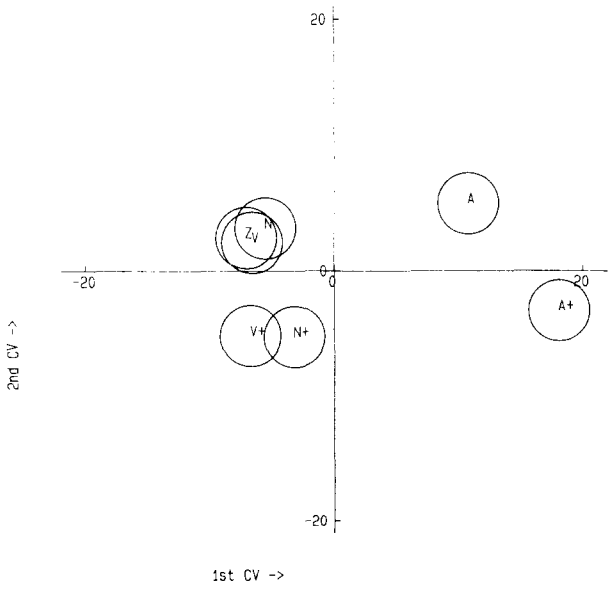

Fig. 3. Canonical variate 1 versus canonical variate 2 for tea at zero time and after 16 and 48 weeks storage (Z, zero time, V, vacuum 16 weeks, N, nitrogen Xushed 16 weeks, A, standard air 16 weeks, V+, vacuum 48 weeks, N+, nitrogen Xushed 48 weeks, A+, standard air 48 weeks).

Table 3. Summary of the results of the discriminant analysis

Volatile	First canonical variate	Second canonical variate
Hexanal	−5·74	−0·48
trans-2-Octenal	57·06	−3·44
trans,cis-2,4-Heptadienal	−10·83	26·90
trans,trans-2,4-Heptadienal	−10·14	36·73
β-Cyclocitral	−12·26	3·85
β-Ionone	−5·80	2·70

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