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PRODUCTION OF VOLATILES BY DEGRADATION OF LIPIDS DURING MANUFACTURE OF BLACK TEA

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Key Word Index—*Camellia sinensis*; Theaceae; tea leaves; mechanical damage; manufacture; lipid breakdown; volatiles; *cis*-3-hexenal; linolenic acid.

Abstract—During manufacture of black tea, lipids are degraded to volatile constituents. *Cis*-3-hexenal was present in appreciable amounts in the various parts of fresh shoots and decreased in the second leaves during manufacture. There was a simultaneous increase in *trans*-2-hexenal. Linalol and methyl salicylate also increased appreciably during rolling and fermentation. Most of the volatiles were lost during the firing process. The above trend was borne out by the 'potential' of the leaves for the production of volatiles as indicated by the increased amounts of volatiles produced by homogenizing the tissue in water against controls homogenized in 0.1 N acid. The C_6-aldehydes present in the headspace of withered shoots increased significantly following mechanical damage. The major fatty acids of the lipids in the various parts of the shoots were linolenic, linoleic, palmitic, oleic and stearic acids. The ratio of linoleic to linolenic acid in the stems was much higher than that of the leaves or buds and this was reflected in its higher 'potential for formation of hexanal. During withering and rolling of the second leaves, the unsaturated fatty acids showed substantial losses compared with the saturated acids. It is suggested that the enzymic breakdown of membrane lipids initiate the formation of volatile carbonyl compounds which are partly responsible for the flavour of black tea.

INTRODUCTION

The manufacture of black tea is a complex biological process which is affected by the extent to which the tea shoots (terminal bud with two leaves attached) are dehydrated and broken down by mechanical means and by enzymic action during processing. Certain marked chemical changes which take place during manufacture are largely responsible for the development of the colour and flavour of the finished product. Sanderson [1] has reviewed the biochemical reactions that occur during manufacture and has shown that certain black tea aroma constituents are formed from amino acids. However, recent investigations on the breakdown of lipoprotein membrane structures in disrupted plant tissues, with the concomitant formation of volatile products having characteristic flavour properties, have served to emphasize the importance of lipids in flavour development [2–9]. It has been shown with tea that there is widespread damage to membrane structures during manufacture [10]. Investigations using macerated tea leaves [4] and chloroplasts isolated from the leaves [11] have shown that an enzyme system in chloroplasts catalyzes the oxidative splitting of linolenic and linoleic acids mainly to *cis*-3-hexenal and *n*-hexanal respectively. The *cis*-3-hexenal formed is easily isomerized to *trans*-2-hexenal.

The purpose of the present investigation was, (a) to confirm the role of unsaturated fatty acids in the production of a number of volatile compounds during manufacture, (b) to show that the unsaturated fatty acid composition of the different tissues can influence the potential for production of volatiles, and (c) to describe a simple method to assess the 'flavour potential' of a clone.

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RESULTS

The main object of the preliminary experiments with material from Cambridge was to determine the type of changes that occur in the different parts of the shoots during storage at 5–6° for two days. The experiments with shoots from Sri Lanka were more comprehensive and aimed at explaining the mechanism for production of volatiles by lipid breakdown.

Preliminary experiments with material from Cambridge

The different parts of the fresh shoots (second leaf, bud and stem) were separated and blended aerobically with distilled H_2O or 0.1 N HCl. The pentane-soluble volatiles produced after 5 min at 25° are shown in Table 1. It is assumed that the volatiles obtained with acid extraction (enzyme inactivation) reflect their relative proportions *in vivo* and the increased amounts of the volatiles obtained using water extraction is a measure of the additional volatiles formed during incubation and indicates the 'potential' of the tissue to produce the volatiles during this period.

The main volatiles present in the acid extracted tissues were *cis*-3-hexenal and linalol, whereas hexanal, *trans*-2-hexenal and *cis*-3-hexenol were present in very small amounts. The amounts of volatiles present in the second leaves were not appreciably altered on storage for a further 24 hr at 6°.

With water extraction of the tissues an increase in all the pentane-soluble volatiles could be clearly observed.

Table 1. Production of pentane-soluble volatiles by different parts of shoots from Cambridge (μg/g tissue)

	Second leaf					Bud		Stem*
Extraction media	0.1 N HCl	0.1 N HCl	H_2O	H_2O	H_2O (Rolled)‡	0.1 N HCl	H_2O	H_2O
No. of hr after plucking	24	48	24	48	24	24	24	24
Volatile compounds†								
n-Hexanal	T	T	4.8	5.8	2.8	T	3.7	7.0
cis-3-Hexenal	30.3	27.6	95.7	72.5	78.0	20.0	57.0	49.4
trans-2-Hexenal	0.7	0.8	2.5	4.2	7.6	0 7	1.9	0.7
cis-3-Hexenol	0.6	0.7	6.0	5.8	24.2	T	8 0	3.3
Linalol	4 2	4.9	17.0	18.7	20.0	2.3	44.2	18.1
Methyl salicylate	T	T	T	T	T	T	T	1.5

* In this and the following Tables stem refers to the included stem of the shoot.
† The compounds are listed in the order in which they are eluted from the column
‡ Second leaf rolled without withering.
T—Trace

The potential of the second leaf to form volatiles was not appreciably altered on storage for a further 24 hr at 6°. When the unwithered second leaf was minced there was a marked increase in the content of *cis*-3-hexenol. These results, in addition to confirming the findings of other workers, showed that useful information on the production of volatiles could also be obtained from shoots air-freighted from Sri Lanka.

Experiments with material from Sri Lanka

Production of volatiles by different parts of the shoots. The volatiles produced by different parts of the shoots are shown in Table 2. The main products obtained on extraction with acid were *cis*-3-hexenal and linalol, there were however relatively low amounts of the other volatiles. The *cis*-3-hexenal content of the stem was much lower than that of the other tissues. Extraction with water gave increased amounts of all the volatiles except *cis*-3-hexenol. Compared with the stems, the leaves have a considerably higher potential for formation of *cis*-3-hexenal and this capacity increased with maturity of the leaves. The stems, however, have a much higher potential for production of hexanal, linalol and methyl salicylate. These results are in general agreement with the preliminary experiments.

Changes in the production of volatiles during manufacture. To minimize the variation between samples, these experiments were carried out with the second leaves (Table 3). With acid extraction, there is a notable decrease in the content of *cis*-3-hexenal during the stages of manufacture. The content of *trans*-2-hexenal however, increased appreciably during withering and rolling and decreased on prolonged fermentation. These findings suggest that there is isomerization of the *cis*-3-hexenal to *trans*-2-hexenal. It is also of interest that the content of linalol and methyl salicylate in pentane extracts increased during manufacture.

The results of the water extraction experiments show that the factors responsible for the production of *cis*-3-hexenal are markedly reduced during withering, rolling and fermentation. However, there was a marked increase in the factors responsible for the production of *trans*-2-hexenal during withering followed by a decrease during rolling and fermentation. The potential for production of linalol increased during withering and decreased thereafter. This is of interest because linalol and its oxides influence the aroma of black tea to a considerable extent [11]. The linalol is probably produced from an 'oxygenated' isoprenoid hydrocarbon. There was a notable increase in the production of methyl salicylate during rolling and fermentation. Very small amounts of volatiles were present in the fired tea showing that most of these had volatilized during the drying process.

Thus the trend in the changes of volatiles during manufacture (reflected by the acid extraction studies) are generally borne out by the 'potential' of the leaves for the production of the volatiles as indicated by the formation of these during incubation of water homogenates.

Headspace analysis. Because very small amounts of the relevant volatiles were present in the headspace of the fresh, withered and fired tea, only those present in the headspace of rolled and fermented shoots are shown in Table 4. Appreciable amounts of three or four compounds having a lower RRT than hexanal were present at all

Table 2. Production of pentane-soluble volatiles by different parts of tea shoots from Sri Lanka (μg/g tissue)

Extraction media	Bud		First leaf		Second leaf		Stem		Shoot
	0.1 N HCl	H_2O	0.1 N HCl	H_2O	0.1 N HCl	H_2O	0.1 N HCl	H_2O	H_2O
Volatile compounds									
n-Hexanal	0.7	1.5	0.44	7.2	0.4	6.5	0.5	13 1	5.9
cis-3-Hexenal	16.6	20.3	19.3	51.3	27.5	206.1	2.5	65 1	57.4
trans-2-Hexenal	0 7	10.5	0.6	18.5	0.6	11.1	0.4	4.8	17 6
cis-3-Hexenol	T	T	T	T	T	T	T	T	T
Linalol	1.3	12.9	2.2	14.7	2.4	7.2	1.5	26 2	7.8
Methyl salicylate	T	1.3	T	T	1.7	T	1.3	23.5	T

Table 3. Production of pentane-soluble volatiles by second leaf during manufacture (μg/g tissue)

	Fresh		Withered		Rolled		Fermented (2 hr)		Fermented (5 hr)		Fired*	
Extraction media	0·1 N HCl	H_2O	0·1 N HCl	H_2O	0·1 N HCl	H_2O	0·1 N HCl	H_2O	0·1 N HCl	H_2O	0·1 N HCl	H_2O
Volatile compounds												
n-Hexanal	0·4	6·5	0·3	6·9	0.6	5·2	0·7	1·4	0·6	1.3	T	T
cis-3-Hexenal	27·5	206·1	4·3	130·9	3·3	29·1	0·6	6·8	0·4	3·4	T	0·5
trans-2-Hexenal	0·5	11·1	2·7	27·7	8·3	18.9	5·8	10·0	3·3	7·7	T	2·0
cis-3-Hexenol	T	T	0·7	T	0·6	T	0·6	T	T	0·8	T	T
Linalol	2·4	7·2	2·1	14.4	5·9	12.2	6·0	9·4	5·5	9·8	T	3·1
Methyl salicylate	1·7	T	T	T	2·8	5.5	8·1	10·5	6·2	9·8	T	3·2

* Fired tea from leaves fermented for 2 hr.

Table 4 Concentration of volatiles in headspace of rolled and fermented shoots* (μg/ml of air)

	Rolled	Fermented (2 hr)
Volatiles		
n-Hexanal	3.1	1.6
cis-3-Hexenal	0.8	0.6
trans-2-Hexenal	2.7	2.1
cis-3-Hexenol	0.2	0.2
Linalol	0.3	0.3
Methyl salicylate	0.3	0.2

* Only the relevant volatiles are listed.

stages of manufacture but these are not included in the Table. From the results it is clear that a marked increase in the volatile compounds, especially *trans*-2-hexenal and hexanal, occurs following mechanical damage. Presumably the *trans*-2-hexenal is formed by isomerization of the *cis*-3-hexenal derived from linolenic acid, whereas the hexanal arises from the linoleic acid and thus both fatty acids are degraded during tea manufacture. The relatively high levels of *trans*-2-hexenal detected by Coggon *et al.* [9] in the headspace of 'fresh leaves' could be due to the fact that leaves stored at −40° were used in the study. When such leaves are warmed to room temperature the enzymic breakdown of lipids is considerably enhanced.

Changes in fatty acids. Table 5 shows the total fatty acid content of the various parts of shoots and the changes in the second leaf during withering and rolling. In the various tissues, the major fatty acids of the lipids in decreasing order of concentration were linolenic, linoleic, palmitic, oleic and stearic acids. These differences in fatty acid composition reflect the differences of maturity and function of the tissues. The fatty acids of major interest in this work are the polyunsaturated acids, which are susceptible to enzymic and chemical oxidations. The ratio of linoleic to linolenic acid in the stems is much higher than that of the leaves or buds and this is reflected in its higher potential for production of hexanal as indicated by hexanal: hexenal ratios.

The fatty acid content of the withered leaves was found to be less than that of the 'fresh leaves' and there was still further loss on rolling. The unsaturated acids (18:1, 18:2 and 18:3) showed substantial losses on withering and rolling compared with the saturated acids (16:0 and 18:0). This order probably reflects the relative conversion of the acids to aldehydes and other compounds. These results on the changes in the fatty acids of tea are in general agreement with those of other workers [2, 13].

DISCUSSION

The characteristic flavour of black tea develops during the fermentation period of manufacture and the reasons for this have not been fully understood. Recent work [5–7] on the production of non-volatile and volatile carbonyl compounds in disrupted plant tissues by the enzymic oxidation of polyunsaturated fatty acids from lipids, has provided additional information on the mode of formation of some of the flavour compounds. Hatanaka and Harada [4] have reported the enzymic formation of *trans*-2-hexenal, *cis*-3-hexenal, hexanal and *cis*-3-hexenol from linolenic and linoleic acids in macerated tea leaves. Confirmatory evidence for this has been obtained by other workers. Our studies show that more comprehensive information could be obtained by determining the 'flavour potential' of different parts of the shoots and relating this to the volatiles present and to the unsaturated fatty acid composition.

The results of this investigation have confirmed that the aldehydes and alcohols found in disrupted tea leaves may be obtained by macerating the various parts of the shoot either alone or with distilled water in the presence of air. In addition to the compounds reported by other workers, we found appreciable amounts of methyl salicylate. The fresh leaves produced significant amounts of *cis*-3-hexenol when the leaves were damaged in air (column 6, Table 1)—a finding which is in agreement with Hatanaka and Harada [4]. This property is appreciably reduced on withering. The potential of the stems for production of hexanal is higher than that of the other parts and this may be due to the fact that its relative

Table 5. Total fatty acid content in lipids of different parts of shoots (μg/g equivalent of fr. wt)

Fatty acids	Stem	Bud	First leaf	Second leaf (fresh)	Second leaf (withered)	Second leaf (rolled)
Palmitic (16:0)	512	1117	971	1078	958	898
Stearic (18:0)	45	134	158	177	175	155
Oleic (18:1)	91	173	208	278	196	158
Linoleic (18:2)	788	1373	1211	1326	1137	1043
Linolenic (18:3)	813	2159	2013	2346	2042	1730

linoleic acid concentration is higher. *Cis*-3-hexenal is the main aldehyde present in all the tissues when they are 'fresh'. It would seem probable that during rolling and fermentation, most of the *cis*-3-hexenal is converted to *trans*-2-hexenal and *cis*-3-hexenol which are readily volatilized.

The acid and water extraction studies of the volatiles of the second leaf during manufacture generally supplement each other. It would therefore appear that the volatiles produced by the latter method is a fair index of the 'potential' of the shoot for the production of flavour compounds. This method requires very small amounts of shoots and may prove to be useful for screening tea clones in breeding experiments.

The levels of unsaturated fatty acids in the different parts of the shoots and the progressive decrease of these acids in the second leaves during withering and rolling would account for the results of the headspace analysis of shoots during manufacture. The marked increase in the levels of C_6-aldehydes in the headspace of rolled shoots and the simultaneous decrease in the amounts of linolenic and linoleic acids indicate that physical disruption of the shoots initiates autolytic changes, catalyzed by endogenous enzymes. Amongst these enzymes are those which cause hydrolytic and oxidative breakdown of lipids [6]. Although isomerization of *cis*-3-hexenal to *trans*-2-hexenal can occur non-enzymically, recent studies have indicated that the rapid isomerization in disrupted plant tissues is catalyzed by an isomerase enzyme (Galliard, T., Reynolds, J., Philips, D. R. and Matthew, J. A. unpublished results). Thus our work supports earlier studies and provides additional information on the fatty acids concerned and on the 'potential' of the different parts of shoots for flavour production.

EXPERIMENTAL

Materials. Fresh shoots (a) In the preliminary experiments, shoots collected from plants grown in a heated greenhouse in the Cambridge Botanic Gardens were used. The shoots were plucked on 24th January 1977. Most of the plants were of the Assam variety (*Camellia sinensis*, var. *assamica*) and were grown in soil in large pots with a nutrient soln. The majority were about 15–16 yr old. The main purpose of these experiments was to find out whether the ability of the shoots to form volatiles was impaired during storage at 5–6° for 2 days. (b) In later experiments, shoots plucked from selected bushes at the Tea Research Institute of Sri Lanka, Talawakelle (1371 6 m) were packed in polythene bags as soon as possible and flown from Sri Lanka to Norwich The samples were kept at 5–6° during transit and used in the laboratory about two days after being plucked. The term 'fresh shoots' is therefore used in a restricted sense, because of this delay; since 'chemical withering' begins immediately after the shoot is separated from the parent plant. All experiments were carried out on shoots from Clone TRI 2024 and were plucked on 1st February 1977. In order to minimize the variation between the different parts of the shoots tested, the manufacture of black tea on a miniature scale was carried out in the laboratory, using only the second leaves. However, for experiments on headspace analysis, whole shoots were used. *Withered leaves.* The leaves were spread on a perforated tray and allowed to wither for 22 hr at 21° During this period the moisture content of the leaves dropped from 73 to 55%. *Rolled leaves.* Withered leaves were minced so that the leaves ruptured as in conventional manufacture. *Fermented leaves.* Minced leaves were spread on a tray and allowed to 'ferment' for 2 and 5 hr respectively. *Fired tea.* 'Fermented leaves' were dried in an oven at 95° for 1 hr to simulate the firing conditions of conventional manufacture. During this period the moisture content of the leaves dropped to 6%.

Preparation of volatile fractions. The tissue (1 g equivalent of fr. material) was blended in a 50 ml glass tube with 10 ml of either H_2O or 0.1N HCl for 1 min in the atmosphere. The triturated material from H_2O extraction was kept for 5 min at 25° in a H_2O bath whereas the acid extracted material was used directly. Pentane (1 ml) was added to the homogenate and the mixture was shaken and centrifuged in screw-topped tubes to effect a separation of the pentane- and water-soluble fractions. The clear pentane layer (~0.5 ml) was removed and 10 µl was analysed by GLC [14]. The identities of the volatiles were established by GC–MS.

Fatty acid analysis. The fatty acids were extracted from 1 g equivalent of fr. tissue using methanolic KOH and estimated as methyl esters by GLC [15].

Headspace volatile analysis. For headspace analysis, the shoots at different stages of manufacture were used. The shoots (60 g equivalent of fr. material) were placed in a 250 ml conical flask (fitted with a capillary tube sealed with silicone rubber discs) and were allowed to equilibrate for 15 min. At the end of this period, 10 ml of the volatiles were withdrawn using a hypodermic syringe and 8 ml were used directly for GLC analysis.

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REFERENCES

1. Sanderson, G. W. (1972) in *Structural and Functional Aspects of Phytochemistry. Recent Advances in Phytochemistry* (Runeckles, V. C. and Tso, T C. eds) Vol. 5, p. 247. Academic Press, New York.
2. Saijyo, R. and Takeo, T. (1972) *Plant Cell Physiol.* **13**, 991.
3. Saijyo, R. and Takeo, T. (1973) *Agr. Biol. Chem.* **37**, 1367.
4. Hatanaka, A. and Harada, T. (1973) *Phytochemistry* **12**, 2341.
5. Gardner, H. W. (1975) *J. Agr. Food Chem.* **23**, 129.
6. Galliard, T. (1975) in *Recent Advances in the Chemistry and Biochemistry of Plant Lipids* (Galliard, T. and Mercer, E. I. eds) p. 319. Academic Press, London.
7. Galliard, T., Matthew, J. A., Fishwick, M. J. and Wright, A. J. (1976) *Phytochemistry* **15**, 1647.
8. Hatanaka, A., Sekiya, J. and Kajiwara, T (1977) *Plant Cell Physiol.* **18**, 107.
9. Coggon, P., Romanczyk, Jr. L. J. and Sanderson, G. W. (1977) *J. Agri. Food Chem.* **25**, 279.
10. Selvendran, R. R. and King, N. R (1976) *Ann. Appl Biol* **83**, 463.
11. Sekiya, J., Numa, S., Kajiwara, T. and Hatanaka, A (1976) *Agr. Biol. Chem.* **40**, 185.
12. Gianturco, M. A., Biggers, R. E. and Ridling, B. H. (1974) *J. Agr. Food Chem.* **22**, 758.
13. Hatanaka, A., Kajiwara, T and Sekiya, J. (1976) *Phytochemistry* **15**, 1889
14. Galliard, T., Phillips, D. R. and Reynolds, J. (1976) *Biochim. Biophys. Acta* **441**, 181.
15. Fishwick, M. J., Wright, A. J. and Galliard, T (1977) *J. Sci. Fd Agric.* **28**, 394.