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LIPID DEGRADATION DURING MANUFACTURE OF BLACK TEA

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Key Word Index—*Camellia sinensis*; Theaceae; tea leaves; mechanical damage; manufacture; lipid breakdown; galactolipids; linolenic acid.

Abstract—About 85% of the fatty acids liberated during the manufacture of black tea can be attributed to autolysis of 4 major polar lipid classes in tea leaf tissue, phosphatidylcholine, monogalactosyldiglyceride, digalactosyldiglyceride and phosphatidylethanolamine. Linolenic, linoleic and palmitic acids are the principal fatty acids released from these lipids and they all undergo further degradation. Linolenic acid (60% of fatty acids released) is derived mainly from galactolipids and thus the upper limit of release is dependent on the chloroplast maturity and content of the leaf tissues. Lipid breakdown is complete after 2 hr fermentation and, as there appears to be no accumulation of long chain fatty acid intermediates, it is probable that volatile production has also ceased at this time.

INTRODUCTION

Plant tissues that undergo mechanical damage release lipid-degrading enzymes [1] which attack lipoprotein membrane structures and/or storage lipids to release fatty acids and these can undergo further degradation. Volatile constituents with characteristic flavour properties may be found amongst these degradation products [2–4]. Polyunsaturated fatty acids have already been identified as precursors of C_6 aldehydes and alcohols in fresh tea leaves [5, 6] and the role of these unsaturated fatty acids in the production of a number of volatile C_6 compounds during tea manufacture has been observed [7].

Details are given in this paper of the fatty acid composition of the tea leaf lipids and of lipid degradation at various stages in black tea manufacture. Knowledge of the distribution of the released fatty acids amongst the lipid classes helps to explain why the various parts of tea shoots differ in flavour potential [7]. The absence of long chain intermediates pinpoints the stage in manufacture during which volatile production must cease due to lack of available fatty acid precursors.

RESULTS AND DISCUSSION

The main fatty acid lost during black tea manufacture is linolenic acid, with smaller amounts of linoleic and palmitic acids (Table 1). These 3 fatty acids account for *ca* 90% of the fatty acids released. The loss of these fatty acids coincides with the degradation (Table 2) of 4 major polar lipids (Table 3): phosphatidylcholine, monogalactosyldiglyceride (MGDG), digalactosyldiglyceride (DGDG) and phosphatidylethanolamine. Comparison of the total estimated release (Table 4) of the 3 main fatty acids from these major lipids with the loss of the same fatty acids from the total lipid (Table 1) shows that the degradation of these major lipids accounts for *ca* 85% of the fatty acids lost in the manufacture of black tea. Whilst MGDG and DGDG together contribute 44% of the released 16:0 and 17% of the released 18:2, they contribute *ca* 85% of the released linolenic acid, the major fatty acid lost during black tea manufacture (Table 4). Table 4 is derived from Tables 2 and 3 and assumes that the acyl hydrolases released on tissue disruption are non-specific in their liberation of fatty acids from the polar lipids [1].

TLC separation of neutral lipids shows no evidence of free fatty acid, fatty acid hydroperoxide or long chain aldehyde accumulation in the extracts.

Lipid degradation (Table 2) is shown to be almost complete after only 2 hr fermentation and, because there is no evidence of long chain fatty acid intermediates, it

Table 1. Fatty acid composition of total lipid from stages in black tea manufacture

Sample	FAMES mg/100 g fr. wt (FAME loss mg/100 g fr. wt relative to 'fresh' sample)						
	Total	16:0	18:0	18:1	18:2	18:3	Others
'Fresh'	683 (—)	158 (—)	38	34	147 (—)	258 (—)	49
Withered	578 (105)	133 (25)	29	26	125 (22)	221 (37)	45
Rolled	550 (133)	141 (17)	31	29	128 (19)	176 (82)	45
Fermented 2 hr	433 (250)	113 (45)	25	23	105 (42)	130 (128)	37
Fermented 5 hr	443 (240)	120 (38)	26	25	106 (41)	124 (134)	42
Fired	415 (268)	110 (48)	22	23	96 (51)	108 (150)	56

Table 2. Percentage polar lipid retained at stages in black tea manufacture relative to 'fresh' sample*

	'Fresh'	Withered	Rolled	Fermented 2 hr	Fermented 5 hr	Fired
Phosphatidylcholine	100	83	77	61	57	49
Monogalactosyldiglyceride	100	74	49	28	27	28
Digalactosyldiglyceride	100	81	62	43	38	38
Phosphatidylethanolamine	100	72	68	52	52	52
Sterol acyl monoglucoside	100	108	†	91	104	88
Phosphatidylglycerol	100	‡	‡	71	70	65
Cerebroside	100	87	91	88	90	100
Sterol monoglucoside	100	95	100	99	102	107

* Lipid analysis by TLC densitometry (see Experimental).
† Interference from neutral lipids.
‡ Transient interference possibly from phosphatidic acid.

may well be that volatile production is also complete at this time. Volatile production appears to be concurrent with fatty acid release, the greater part occurring during rolling and the initial period of fermentation.

The breakdown of the large amounts of galactolipid present in tea leaf chloroplasts accords with other work [8], which has shown widespread chloroplast degradation after tea leaf tissue has been rolled. As the majority of the linolenic acid released is derived from galactolipids present in tea leaves, it is possible that chloroplast maturity and content determine the flavour potential of different parts of tea shoots as will their potential degradative enzymic activities. Previous studies [7] have indicated that the major proportion of C_6 volatiles from tea leaf tissue can be derived from a linolenic acid precursor.

The enzymic pathways between linolenic and linoleic acids and volatile C_6 aldehydes and alcohols in tea leaves have not yet been clarified. It has been suggested [9–11] that C_6 volatiles arise by a direct cleavage of enzymically released fatty acids, although results with leaves of *Phaesolus vulgaris* [12] have shown that, as in tomato [13, 14] and cucumber fruit [3, 4, 15, 16], fatty acid hydroperoxide intermediates are directly involved in the conversion of polyunsaturated fatty acids to volatile carbonyl compounds.

EXPERIMENTAL

Materials. The manufacture of black tea on a micro scale was carried out in the laboratory as described in ref. [7] using only the second leaves of 'fresh' shoots (Clone TRI 2024) that had been flown in from Sri Lanka [7]. 'Fresh' shoots are *ca* 24–48 hr old and the term is therefore used in a restricted sense.

Samples. 'Fresh'; withered 22 hr at 21°; rolled; fermented 2 hr; fermented 5 hr; fired 95°, 1 hr. Only one sample of each was provided for analysis because of the limited amount of material available.

Lipid extraction. Tissue samples (5 g equivalent of fr. material) were killed by refluxing in *iso*-PrOH and then extracted as previously described [17, 18] and partially purified by partitioning with 300 ml $CHCl_3$–MeOH–H_2O (8:4:3) [19].

Lipid analysis. Total fatty acids were estimated in duplicate as fatty acid Me esters (FAMES) [17]. TLC densitometry of the polar lipids was as previously described [13]. TLC of neutral lipids was performed by developing 20 cm plates in Et_2O–C_6H_6–EtOH–HOAc (80:100:4:0.4) to a distance of 13 cm, drying under N_2 and re-running to full distance with Et_2O–hexane (1:19). A portion of the crude lipid from the 'fresh' tea sample was purified from non-lipid contaminants [20] and divided into a neutral plus glycolipid and a phospholipid fraction by chromatography on a Si gel column [21]. Individual lipids from these fractions were separated by TLC and quan-

Table 3. Lipid and fatty acid composition of purified 'fresh' leaf lipid

	FAME mg/100 g fr. wt	(% total)	Fatty acid (% peak area) 16:0	18:0	18:1	18:2	18:3	Others
Phosphatidylcholine	140.0	(20.5)	24.2	4.3	7.0	41.1	23.0	0.4
Monogalactosyldiglyceride	115.0	(16.8)	4.3	1.1	1.0	4.9	88.3	0.4
Digalactosyldiglyceride	92.6	(13.6)	22.0	7.1	3.3	5.9	60.9	0.8
Sulpholipid	45.4	(6.6)	41.9	4.4	6.8	7.8	38.6	0.5
Phosphatidylethanolamine	44.5	(6.5)	22.1	3.4	5.3	46.8	18.8	3.6
Sterol acyl monoglucoside	26.9	(3.9)	33.1	15.5	4.6	17.9	23.9	5.0
Phosphatidylglycerol	24.4*	(3.7)	29.7	2.8	17.5	16.2	14.3	19.5
Phosphatidylinositol	22.3	(3.3)	35.9	5.9	5.1	27.9	24.5	0.7
Trigalactosyldiglyceride	15.9	(2.3)	33.8	6.4	4.8	24.7	29.1	1.2
Cerebroside	6.6†	(1.0)	17.0	5.8	4.9	21.0	38.2	13.1
Neutral lipids	113.0	(16.5)	36.0	13.1	6.6	11.1	8.4	24.8
Others	35.4	(5.2)						
Total	683.0	(100.0)						

* Contains 19.5% 16:1 *trans* 3.
† Contains 12% 2-OH 16:0.

Table 4. Estimated* loss of fatty acids on breakdown of major lipids at stages in black tea manufacture relative to 'fresh' sample

	Estimated FAME loss mg/100 g fr. wt														
	Withered			Rolled			Fermented 2 hr			Fermented 5 hr			Fired		
	16:0	18:2	18:3	16:0	18:2	18:3	16:0	18:2	18:3	16:0	18:2	18:3	16:0	18:2	18:3
Phosphatidylcholine	5.8	9.8	5.5	7.8	13.2	7.4	13.1	22.3	12.5	14.6	24.8	13.9	17.2	29.2	16.4
Monogalactosyldiglyceride	1.3	1.5	26.1	2.5	2.9	52.2	3.6	4.1	73.4	3.6	4.1	73.8	3.5	4.0	72.8
Digalactosyldiglyceride	4.0	1.1	11.0	7.7	2.1	21.4	11.6	3.1	32.0	12.6	3.4	34.8	12.6	3.4	34.8
Phosphatidylethanolamine	2.7	5.8	2.3	3.2	6.7	2.7	4.7	10.0	4.0	4.7	10.0	4.0	4.7	10.0	4.0
	13.8†	18.2	44.9	21.2	24.9	83.7	33.0	39.5	121.9	35.5	42.3	126.5	38.0	46.6	128.0

* Table constructed from data contained in Tables 2 and 3.

† Estimated total loss of individual fatty acids from the 4 lipids can be compared to total FAME loss from all lipids as shown in Table 1.

titatively estimated as FAMES [17] prepared in the presence of Si gel removed from the plate.

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REFERENCES

1. 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.
2. Tressl, R., Holzer, M. and Apetz, M. (1975) *Proc. Int. Symp. Aroma Research*, Zeist 1975. Pudoc, Wageningen.
3. Phillips, D. R. and Galliard, T. (1977) *Phytochemistry* **17**, 355.
4. Phillips, D. R., Matthew, J. A., Reynolds, J. and Fenwick, G. R. (1979) *Phytochemistry* **18**, 401.
5. Hatanaka, A. and Harada, T. (1973) *Phytochemistry* **12**, 2341.
6. Sekiya, J., Numa, S., Kajiwara, T. and Hatanaka, A. (1976) *Agric. Biol. Chem.* **40**, 185.
7. Selvendran, R. R., Reynolds, J. and Galliard, T. (1978) *Phytochemistry* **17**, 233.
8. Selvendran, R. R. and King, N. R. (1976) *Ann. Appl. Biol.* **83**, 463.
9. Major, T. R. and Thomas, M. (1972) *Phytochemistry* **11**, 611.
10. Hatanaka, A., Kajiwara, T. and Sekiya, J. (1976) *Phytochemistry* **15**, 1125.
11. Hatanaka, A., Sekiya, J. and Kajiwara, T. (1977) *Plant Cell Physiol.* **18**, 107.
12. Matthew, J. A. and Galliard, T. (1978) *Phytochemistry* **17**, 1043.
13. Galliard, T., Matthew, J. A., Wright, A. J. and Fishwick, M. J. (1977) *J. Sci. Food Agric.* **28**, 863.
14. Galliard, T. and Matthew, J. A. (1977) *Phytochemistry* **16**, 339.
15. Galliard, T., Phillips, D. R. and Reynolds, J. (1976) *Biochim. Biophys. Acta* **441**, 181.
16. Galliard, T. and Phillips, D. R. (1976) *Biochim. Biophys. Acta* **431**, 278.
17. Fishwick, M. J. and Wright, A. J. (1977) *Phytochemistry* **16**, 1507.
18. Bligh, E. G. and Dyer, W. J. (1959) *Can. J. Biochem. Physiol.* **37**, 911.
19. Folch, J., Lees, M. and Standley, G. H. S. (1957) *J. Biol. Chem.* **226**, 497.
20. Wuthier, R. E. (1966) *J. Lipid Res.* **7**, 558.
21. Rouser, G., Kritchevsky, G., Simon, G. and Nelson, G. J. (1967) *Lipids* **2**, 37.