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SEASONAL VARIATIONS IN *TRANS*-2-HEXENAL AND LINOLENIC ACID IN HOMOGENATES OF *THEA SINENSIS* LEAVES

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Key Word Index—*Thea sinensis*; Theaceae; tea; seasonal variations; *cis*-3-hexenal; *trans*-2-hexenal; *cis*-3-hexenol; linolenic acid.

Abstract—The seasonal variations in the amounts of C_6-volatile components (*cis*-3-hexenal, *trans*-2-hexenal, *n*-hexanal) and their precursors (linoleic and linolenic acid) in homogenates of *Thea sinensis* leaves were quantitatively analyzed throughout the year. Formation of *trans*-2-hexenal began in the middle of April and reached a maximum during July. Towards autumn the aldehyde gradually decreased and, in winter (December to March), was virtually absent. The levels of *cis*-3-hexenol remained constant during May–December. *cis*-3-Hexenal showed a similar variation pattern to that of *trans*-2-hexenal. The major fatty acids in the leaves were palmitic, palmitoleic, oleic, linoleic and linolenic acid, and occurred in non-ionic lipids and phospholipid fractions. The amounts of linoleic and linolenic acid did not show any marked variation except for a big peak in October.

INTRODUCTION

We have been studying the biosynthetic pathway of *cis*-3-hexenol (leaf alcohol) and *trans*-2-hexenal (leaf aldehyde) *via* the intermediate, *cis*-3-hexenal, from linolenic acid in homogenates of *Thea sinensis* leaves [1–11]. Using linolenic [U-^{14}C] and linoleic [U-^{14}C] acid we recently found that *cis*-3-hexenal, *trans*-2-hexenal and *n*-hexanal, were generated by an enzyme bound to lamellae of chloroplasts in tea leaves, when the leaves were cut or mechanically ruptured in the presence of O_2 [11].

To demonstrate the formation of the C_6-volatile components from linoleic and linolenic acid in homogenates of tea leaves throughout the year, the seasonal amounts of C_6-volatile compounds and unsaturated fatty acids were quantitatively analyzed in the period 1974–1976.

RESULTS AND DISCUSSION

Variations in the amounts of C_6-unsaturated aldehydes and the corresponding alcohols

Fresh tea leaves were blended in air, the homogenates steam-distilled and the distillates extracted with Et_2O; the crude essential oil thus obtained was quantitatively analyzed by GLC. The generation of *cis*-3-hexenal was followed by headspace vapour analysis according to the methods previously reported, as this aldehyde is easily isomerized to *trans*-2-hexenal in homogenates [1, 4]. The most prominent seasonal variations in the amounts of C_6-aldehydes took place in the young leaves; the amount of *trans*-2-hexenal formed decreased from autumn to winter, after reaching a maximum in June–July (Fig. 1) During winter, the amount of *trans*-2-hexenal formed was extremely small and, in particular, was not appreciable from the end of December to the middle of April. The seasonal variation pattern in the generation of *cis*-3-hexenal was similar to that of *trans*-2-hexenal (Fig. 2) and the amount of *n*-hexanal was smaller than that of *cis*-3-hexanal throughout the year. On the other hand, the amount of *cis*-3-hexenol formed was less than that of *trans*-2-hexenal throughout the year and showed only minor changes during May–December (Fig. 1).

Variation in the amounts of linoleic and linolenic acid

The results of the quantitative analysis of fatty acids constituting the non-ionic and phospholipid fractions in tea leaves are summarized in Table 1. The major fatty acids in the lipids were palmitic, palmitoleic, oleic, linoleic and linolenic acid, and they were not found in the free fatty acid fraction [12]. Of these acids linolenic acid was by far the most abundant. Linoleic and linolenic acids were more plentiful in the non-ionic fraction than in the phospholipid fraction, and unlike the C_6-aldehydes did not show marked seasonal changes. The concentration of these acids was higher in the non-ionic lipid than in the phospholipid fraction throughout the year

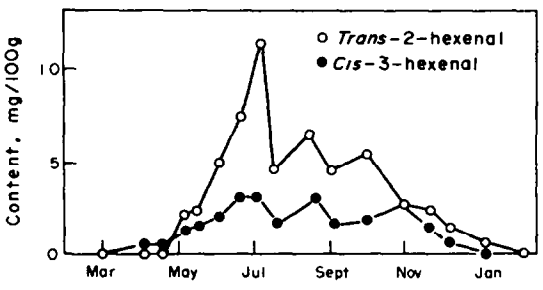

Fig. 1. Seasonal variation of volatile components in homogenates of fresh tea leaves.

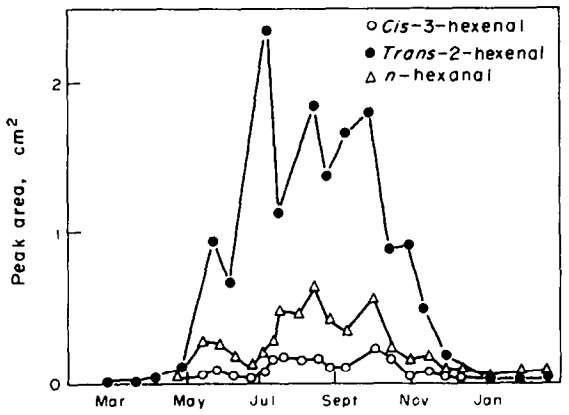

Fig. 2. Seasonal variation of volatile components in homogenates of fresh tea leaves.

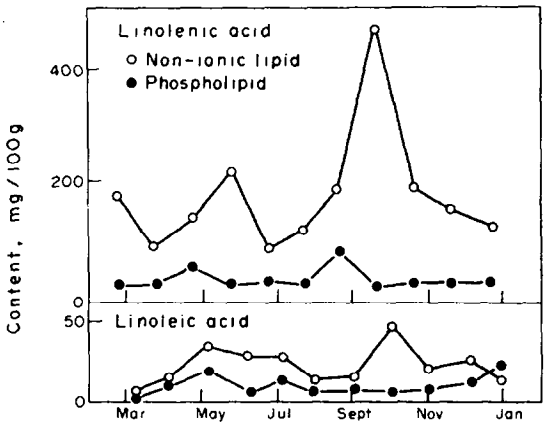

Fig. 3. Seasonal variation of linolenic and linoleic acids in non-ionic and phospholipid fractions in homogenates of tea leaves.

(Fig. 3). The levels of these fatty acids in both the non-ionic and phospholipid fractions notably decreased, corresponding to production of the flavour components on maceration or blending in the presence of O_2. The decrease in linolenic acid was large in the early summer and small in winter (Table 2). These variations in the decrease in unsaturated fatty acids during blending corresponded to the seasonal variations in the generation of C_6-unsaturated aldehydes (Figs. 1 and 2).

The C_6-aldehyde forming activities reached a maximum in July–August and were very slight or absent during January–March, paralleling the seasonal variations of C_6-volatile components in the leaves [13].

EXPERIMENTAL

Fresh leaves of *Thea sinensis* var. *Yabukita* grown in Yamaguchi University were harvested from May 1974 to March 1976. Linolenic and linoleic acids were high-purity grade of above 98%. Authentic specimens of *n*-hexanal, *cis*-3-hexenal and *trans*-2-hexenal were synthesized through unequivocal routes [6].

Preparation of essential oil. Fresh leaves (150 g) were blended with H_2O (500 ml) for 3 min in air, and the homogenate was steam-distilled to give 500 ml of distillate. Distillate was extracted with 3 × 50 ml of Et_2O after saturation with NaCl and the combined extracts dried. Evaporation of the solvent afforded 20 mg of crude essential oil. A portion of the oil was quantitatively analyzed by FID GLC using a 3 mm × 3 m stainless steel column packed with 20% PEG-20 M on Celite 545; column temp, 120°C; injection temp, 150°; flow rate N_2, 80 ml/min.

Preparation of vapour sample. Fresh leaves (5 g) were blended for 2 min with H_2O (80 ml) in air, and the homogenate was transferred immediately to a rubber-stoppered conical flask (50 ml) and incubated at 25° for 5 min, then at

Table 1. Composition of fatty acids in lipid fractions from tea leaves

Fatty acid	Free fatty acid	Fraction Non-ionic lipid	Phospholipid	Total
Palmitic	trace	38.7*	14.5	53.2
Palmitoleic	trace	5.8	trace	5.8
Oleic	trace	5.0	3.4	8.4
Linoleic	trace	18.5	13.8	32.3
Linolenic	trace	164	8.4	172

* mg/100 g fresh tea leaves, sample in June, 1975.

Table 2. Changes in linoleic and linolenic acid in tea leaves during blending

Fraction	Linoleic acid 0*	Linoleic acid 3	Linolenic acid 0	Linolenic acid 3
Free fatty acid	trace trace	trace trace	trace trace	trace trace
Non-ionic lipid	55.6† 18.5‡	23.5 15.8	218.3 164.0	99.3 145.8
Phospholipid	16.8 13.8	13.3 3.5	11.5 8.4	10.5 1.4
Total lipid	72.4 32.4	36.8 19.3	229.8 172.4	109.8 147.2

* Blending time (min). Zero blending time: fractions prepared from leaves inactivated by heating at 80° for 15 min. † Sampled on the 6th June. ‡ Sampled on the 26th November.

40° for 10 min. 6 ml of headspace volatile thus obtained was analyzed by GLC under the above conditions.

Preparation of fatty acids. Crude lipid was extracted with $CHCl_3$–MeOH (2:1) 1 l. from blended tea leaves (200 g). Fatty acids from each lipid fraction were prepared according to the method of ref. [11] and were converted to Me esters with CH_2N_2–Et_2O. Samples of the esters were analyzed by GLC with 20% PEG-adipate on Chromosorb W, column temp, 180°.

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