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CHANGES IN LIPOXYGENASE ACTIVITY IN RELATION TO LIPID DEGRADATION IN PLUCKED TEA SHOOTS

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Key Word Index—*Camellia sinensis*; Theaceae; lipoxygenase; changes in enzyme activity; peroxide in tea shoots.

Abstract—The activity of lipoxygenase in tea leaf increased after plucking of tea shoots. The changes of lipoxygenase activity were accelerated by the dehydration of tea leaves. The activity of lipoxygenase was higher in the good fermenting clones than in the poor fermenting clones of black tea. The content of peroxide in tea shoots increased with rising lipoxygenase activity.

INTRODUCTION

It is known that the degradation of lipoprotein membranes and/or storage lipids and the production of volatile compounds with characteristic aroma properties are induced by withering or rolling of tea shoots in black tea manufacture [1, 2]. Polyunsaturated fatty acids released from lipids have already been identified as precursors of C-6 aldehydes and alcohols [3–5]. Furthermore, there are many reports on the properties of the enzyme concerned in the formation of C-6 compounds [6–9]. In this paper, the activity and clonal characteristics of lipoxygenase (EC 1.13.11.12), which is concerned in hexenal formation in tea shoots, are described.

RESULTS AND DISCUSSION

Clonal characteristics and the activity of lipoxygenase

The activity of lipoxygenase (LPO) in fresh tea shoots showed some clonal characteristics (Table 1). The activities of LPO in clones used for making high quality black tea, such as Benihomare and Benikaori (Assam-China hybrid), were higher than those in the poor fermenting clone Hatzumomiji (Assam-China hybrid). Furthermore, low activities of LPO have been uniformly obtained from the China clones used in Japan. These variations in LPO activity between tea clones are very similar to those reported earlier in the activity of polyphenol-oxidase in tea leaf [10].

Table 1. Clonal characteristics on lipoxygenase activity

Clone	Variety	Activity (± s.d.)
Hatzumomiji	Assam × China	0.168 ± 0.008*
Satzumabeni	Assam × China	0.232 ± 0.009
Himemidori	China	0.181 ± 0.007
Yabukita	China	0.216 ± 0.011
Asagiri	China	0.232 ± 0.012
C-3	China	0.288 ± 0.015
C-7	China	0.296 ± 0.014
Benihomare	Assam × China	0.328 ± 0.016
Benikaori	Assam × China	0.400 ± 0.022

Activity: O_2 μg/fr. g/min. The average values of three samples. s.d. = standard deviation. Season, May.

Lipoxygenase activity in different parts of a tea shoot

The activity of LPO per unit weight of fresh leaves increased with the maturity of the tea leaves (Table 2). The amounts of hexenal produced by different parts of tea shoots were in general agreement with earlier data [1]. The specific activities of LPO per unit protein were maintained at about the same level in each part of the shoot. Those based on unit chlorophyll, however, decreased with maturity of the leaves.

Hatanaka has reported that the LPO of tea leaf is located on the lamellae fraction of the chloroplast [7]. From the above results, it is clear that the biosynthesis of LPO in tea

Table 2. Lipoxygenase activity in different parts of a tea shoot

Part of shoot	Activity*	Specific activity	
		Protein†	Chlorophyll‡
Bud and 1st leaf	0.116	0.076	0.97
2nd leaf	0.166	0.059	0.82
3rd leaf	0.194	0.074	0.61
4th leaf	0.203	0.088	0.50
5th leaf	0.185	0.080	0.39

* Activity, O_2 μg/fr. g/min. The activities were the average values of two samples from each part; deviations were less than 10%.

† Specific activity based on protein content in the crude enzyme solution, O_2 μl/mg N.

‡ Specific activity based on chlorophyll content, O_2 μl/mg chlorophyll.

Clone, Yabukita. Season, May.

Table 3. Changes in lipoxygenase activity in tea shoots

	Withering (hr)				
	0	2	4	6	8
Non-withered leaves at 5°	0.080* (100)†	0.080 (100)	0.080 (100)	0.080 (100)	0.080 (96)
Non-withered leaves at 25°	0.080 (100)	0.080 (100)	0.080 (100)	0.100 (100)	0.144 (100)
Withered leaves at 25°	0.080 (100)	0.080 (87)	0.112 (85)	0.128 (83)	0.170 (75)

* Activity, O_2 μg/fr. g/min. The activities were the average of two samples from each fraction; deviations were less than 10%.
† Fr. wt of each fraction before and after withering (g).
Clone, Yabukita. Season, May.

leaf depends on the biosynthesis of chloroplast protein, but not on the amount of chlorophyll present.

Changes in lipoxygenase activity in plucked tea shoots

After plucking tea shoots, the activity of LPO in tea leaf was increased during the withering stage of black tea manufacture (Table 3). The rise in LPO activity was depressed in leaves kept at 5°. However, the change in LPO activity in tea leaves withered under incandescent lights was not effectively accelerated in spite of the increase in leaf temperature. Furthermore, the activity of LPO in tea shoots, kept fresh by dipping the stems in water, was maintained at a low level for a long period at room temperature. From these results, it is apparent that the rise of LPO activity in tea leaves might be caused by the dehydration of tea leaves.

Changes in peroxide content in plucked tea shoots

The changes in peroxide content in plucked tea shoots are shown as TBA values in Table 4. The TBA values in the shoots increased during the withering process at room temperature. However, the increase of the TBA value was depressed at 5°. These results coincide with the rise in LPO activity in tea shoots at the withering stage. From these results, it is suggested that the concentrations of peroxide produced by the oxidation of polyunsaturated fatty acids might be increased with the rise of LPO activity in tea shoots.

EXPERIMENTAL

Plant materials. Fresh shoots (*Camellia sinensis*) were plucked from selected bushes at the Tea Research Institute. The portion of two leaves and a bud in a shoot was used in each experiment. Withered leaves: the shoots were spread on a perforated tray and allowed to wither for 8 hr at 25°. Non-withered leaves: the stem portions of the shoots were dipped in water and stood for 8 hr at 25° and 5°.

Preparation of lipoxygenase. A crude enzyme extract was prepared by modifying Hatanaka's method [4]. The shoots (10 g; fr. wt) were homogenized in a mixer for 3 min in 8 vol. of twice diluted McIlvine's buffer, pH 6.1, containing 0.4 M sucrose and 7 g Polyclar AT. The homogenate was filtered through 3 layers of gauze. The chloroplast fraction was precipitated between 1000 *g* and 5000 *g*. The chloroplast fraction was washed with the above-mentioned buffer without Polyclar AT and suspended in 5 ml of the buffer. The chloroplast suspension was used as the crude enzyme solution of lipoxygenase.

Table 4. Changes in TBA value during incubation.

Incubation (hr)	0	24	48
Low (5°) temperature	0.43	0.43	0.45
Room (25°) temperature	0.45	0.88	0.55

TBA value = $A_{530\,nm}$. TBA values were the average of two samples from each fraction; deviations were less than 10%.
Clone, Yabukita. Season, May.

Assay of lipoxygenase activity. The enzyme activity was determined by a polarographic method in a 5 ml reaction mixture containing 1.4 μmol linolenic acid, 0.1% Tween-20 and enzyme soln in twice diluted McIlvine buffer, pH 6.1 [7].

Assay of TBA value (peroxide value). The shoots (1 g fr. wt) were homogenized in a mortar with 5 ml 0.9% KCl. The TBA value in the homogenate was determined by the method of Dahle [11].

Preparation of volatile fractions. The volatile fraction in the shoots was prepared by the method of Selvendran [1].

Assay of protein and chlorophyll. The content of protein nitrogen was determined by the micro-Kjeldahl method. The chlorophyll content in the crude enzyme was calculated from absorbance at 660 and 642.5 nm of the acetone extract, by using the Comar equation [12].

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