# Antidiscoloring Activity of Green Tea Polyphenols on *β*-Carotene

Laura Unten, Mamoru Koketsu,* and Mujo Kim

Central Research Laboratories, Taiyo Kagaku Company, Ltd., 1-3 Takaramachi, Yokkaichi, Mie 510, Japan

The antidiscoloring activity of green tea polyphenols and catechins on *β*-carotene was investigated. The residual ratio of *β*-carotene added in beverages and margarine was measured. Green tea polyphenols showed dose-dependent antidiscoloring activity of *β*-carotene on both the beverage and the margarine. The antidiscoloring effect of (−)-epigallocatechin 3-gallate (EGCg) and L-ascorbate (Vc) in a buffer solution (pH 4.0) containing *β*-carotene was compared under fluorescence irradiation. At 1000 *μ*M, EGCg and Vc maintained residual *β*-carotene amounts of 85.4% and 78.5% and at 100 *μ*M 79.6% and 2.9%, respectively. The strength of the antidiscoloring activity of the catechins, gallic acid (GA), and Vc was as follows: (−)-gallocatechin 3-gallate, (−)-epigallocatechin, EGCg, (+)-gallocatechin ≫ GA > Vc, (−)-epicatechin, (+)-catechin. It was suggested that the hydroxyl group at the 5′-position of the B ring of the catechin skeleton mostly contributed to the antidiscoloring activity.

**Keywords:** *Green tea; catechins; β-carotene; antidiscoloring activity*

## INTRODUCTION

There is an increasing interest in the biological, physiological, and pharmaceutical effects of green tea polyphenols. They are known to inhibit carcinogenesis (Hirose et al., 1994; Zhi et al., 1994) and have been shown to be efficient antioxidants, scavenging oxygen radicals (Yen and Chen, 1995). *β*-Carotene is now known for biological functions such as an anticancer effect (Appel and Woutersen, 1996; Sarkar et al., 1995; Ziegler et al., 1996) and health benefits (Baranowitz et al., 1996; Kohlmeier and Hastings, 1995; Moriguchi et al., 1996) . Additionally, *β*-carotene is also widely used as a coloring agent for beverages and foods. Due to its 11 pairs of double bonds, which are extremely sensitive to oxidation, *β*-carotene discolorizes very easily. *β*-Carotene is extremely susceptible to free radical mediated oxidation. Thus, control of the degradation and discoloration of *β*-carotene in beverages and foods is absolutely desirable. Several studies have been reported on the antioxidative activity of green tea polyphenols (Chen and Tappel, 1995; Kajimoto et al., 1969; Lea and Swoboda, 1957; Matsuzaki and Hara, 1985), but study of the suppression of the discoloration of *β*-carotene by using green tea polyphenols has been mostly neglected. Although Amarowicz and Shahidi (1995) reported antioxidative activity of tea catechins in a *β*-carotene−linoleate model system, the relationship between the antioxidative effect of catechins and their structures was not clarified.

In the present study, the antidiscoloring activity of the green tea polyphenols and individual catechins on *β*-carotene is investigated and the structure−activity relationship of catechin derivatives is discussed.

## MATERIALS AND METHODS

**Materials.** Green tea polyphenols were prepared as reported. The green tea polyphenols consisted of 27.1% (−)-epigallocatechin 3-gallate (EGCg), 19.3% (+)-gallocatechin (GC), 16.7% (−)-gallocatechin 3-gallate (GCg), 16.1% (−)-epigallocatechin (EGC), 8.1% (−)-epicatechin 3-gallate (ECg), 7.5% (−)-epicatechin (EC), and 5.2% (+)-catechin (C). EGCg, GCg, ECg, EGC, GC, EC, and C from green tea polyphenols were prepared according to the reported procedure (Koketsu and Satoh, 1997; Sakanaka et al., 1989, 1996). *β*-Carotene was obtained from Wako Pure Chemicals Co., Ltd. (Osaka, Japan). Water-soluble *β*-carotene powder, which contains 1.5% *β*-carotene, 0.1% tocopherol, and 4.7% gum arabic, was purchased from Sankyo Co., Ltd. (Tokyo, Japan).

**Antidiscoloring Examination of *β*-Carotene Using Beverages.** The antidiscoloring effect of tea polyphenols was examined using beverages. The beverages employed were prepared by mixing the following ingredients: water-soluble *β*-carotene powder (0.04%; 6 ppm as *β*-carotene), ethanol (2%), sugar syrup (20.0%), citrate (1.0%), water (77%), and antioxidant (0−0.1%). The beverages were kept at room temperature and were subsequently measured at 466 nm with a spectrophotometer to estimate the residual *β*-carotene.

**Antidiscoloring Examination of *β*-Carotene Using Margarine.** The antidiscoloring effect of tea polyphenols on margarine containing 50 ppm of *β*-carotene was also examined. Margarine with or without tea polyphenols was exposed to UV radiation set up at 254 nm, to let the autoxidation take place. The oxidation index of margarine samples was evaluated by estimating the white and yellow color density of its surface a using colorimeter (Color Ace Model TC-1, Tokyo Denshoku Co., Ltd., Japan).

**Comparative Study between the Effects of EGCg and L-Ascrobate (Vc).** EGCg and Vc of various concentrations were added to 0.1 M citrate−0.2 M phosphate buffer (pH 4.0) containing 200 ppm of water-soluble *β*-carotene powder (3 ppm as *β*-carotene). To calculate the residual ratio of *β*-carotene, the mixture was irradiated to fluorescence at 30 °C and was measured at 466 nm on day 7.

EGCg and Vc of 50 *μ*M concentration were added to the buffer (pH 4.0) containing 3 ppm of *β*-carotene. The mixture was incubated under the same condition as described above, and the residual ratio of *β*-carotene was subsequently calculated by measuring the UV absorbance of the mixture at 466 nm.

**Antidiscoloring Effect of Individual Catechins on *β*-Carotene.** EGCg, GCg, ECg, EGC, GC, EC, C, gallic acid, and Vc of 50 *μ*M were added to the buffer (pH 4.0) containing 3 ppm of *β*-carotene. The mixture was incubated under the same condition as described above, and the residual ratio of *β*-carotene was subsequently calculated by measuring the UV absorbance of the mixture at 466 nm.

* Address correspondence to this author at the Department of Chemistry, Faculty of Engineering, Gifu University, 1-1 Yanagido, Gifu 501-11, Japan [telephone +81-58-293-2619; fax +81-58-230-1893; e-mail koketsu@apchem.gifu-u.ac.jp].

S0021-8561(96)00752-2 CCC: $14.00 

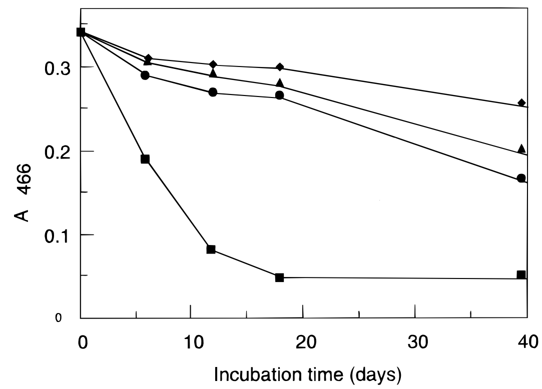


**Figure 1.** Antidiscoloring activity of tea polyphenols on $\beta$-carotene in beverages: ■, control; ●, 50 ppm of L-ascorbate; ▲, 5 ppm of tea polyphenols; ◆, 10 ppm of tea polyphenols.

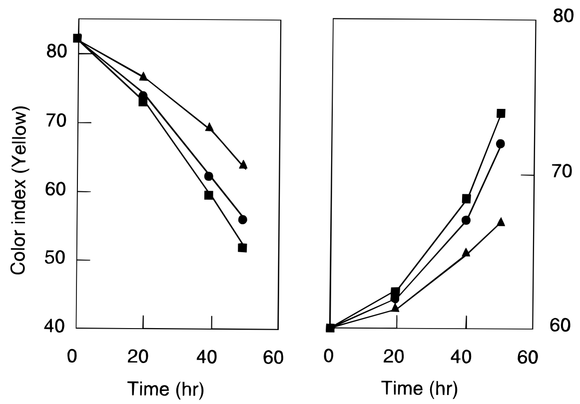


**Figure 2.** Antidiscoloring effect of tea polyphenols on $\beta$-carotene in margarine: ■, control; ●, tea polyphenols at 20 ppm; ▲, tea polyphenols at 40 ppm.

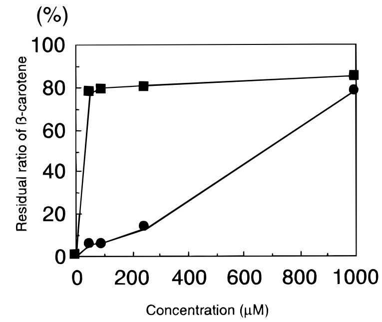


**Figure 3.** Dose-dependent antidiscoloring effect of EGCg and Vc at 7 days: ■, EGCg; ●, Vc.

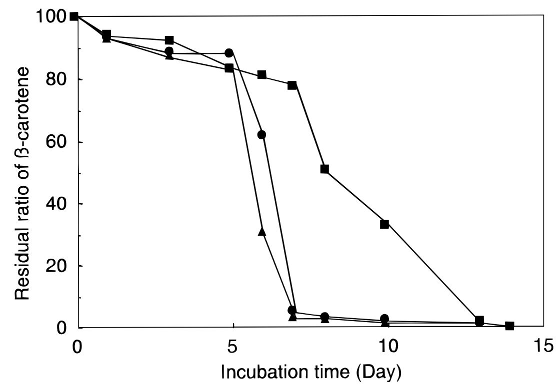


**Figure 4.** Time-dependent antidiscoloring effect of EGCg and Vc at 50 $\mu$M: ■, EGCg; ●, Vc; ▲, control.

## RESULTS

**Antidiscoloring Effect of Tea Polyphenols on $\beta$-Carotene Added in Beverages.** The antidiscoloring effect of green tea polyphenols on $\beta$-carotene was investigated using beverages with or without antioxidant. Tea polyphenols suppressed discoloration of $\beta$-carotene strongly as compared with Vc and the control. The UV absorbance of the control group dropped at a faster rate to 0.08 after 10 days, whereas at 10 ppm of the polyphenol group, this rate was slower, retaining 0.30 after 12 days and 0.25 after 40 days, respectively (Figure 1). The tea polyphenols showed much stronger antidiscoloring effect than Vc.

**Antidiscoloring Effect of Tea Polyphenols on $\beta$-Carotene Added in Margarine.** The antidiscoloring effect of green tea polyphenols on $\beta$-carotene was also investigated using margarine. The yellow color index values of the margarine surface of 40 ppm, 20 ppm of tea polyphenols, and control at 50 h were 64, 56, and 52, respectively, while the white color index values of the same were 67, 72, and 74, respectively. The margarine containing tea polyphenols was found to retain the yellow color. It was indicated that tea polyphenols are effective to protect the discoloration of $\beta$-carotene by UV radiation in margarine (Figure 2).

**Antidiscoloring Effect of EGCg and Vc on $\beta$-Carotene.** EGCg is the most abundant catechin among tea catechins, and its content in this tea polyphenols was found to be 27.1%. Vc is widely used as a water-soluble antioxidant for food. To compare the antidiscoloring effect of EGCg with that of Vc with respect to concentration, several concentrations were tested and sequential alteration of $\beta$-carotene was measured as seen in Figure 3. High $\beta$-carotene contents of 77.8% and 79.6%, respectively, were retained by 50 and 100 $\mu$M EGCg; on the other hand, only 5.4% and 6.1% of $\beta$-carotene content could be retained by 50 and 100 $\mu$M Vc. Thus, to achieve a similar content of 78.5% retention of $\beta$-carotene, about 20 times higher concentration of Vc, i.e. 1000 $\mu$M, is necessary (Figure 3). Figure 4 shows the time-dependent antidiscoloring effect of EGCg and Vc at 50 $\mu$M. The residual ratio of $\beta$-carotene of 50 $\mu$M EGCg or 50 $\mu$M Vc was measured sequentially. The effect of Vc was almost the same as the control: discoloration of $\beta$-carotene progressed gradually until 5 days, after 6 days retained 62.2%, and was completely discolored in 7 days. The discoloration of $\beta$-carotene in the solution with added EGCg progressed gradually until 6 days; 77.8% and 50.9% were retained after 7 and 8 days, respectively (Figure 4).

**Antidiscoloring Effect of Individual Catechins on $\beta$-Carotene.** Table 1 depicts the structure–activity relationship on the antidiscoloring effect of six kinds of catechins, gallic acid (GA), and Vc. The strength of their antidiscoloring activity was as follows: GCg, EGC, EGCg, GC ≫ GA > Vc, EC, C. After 7 days, GCg, EGC, EGCg, and GC retained >69% of their $\beta$-carotene contents and >32% after 10 days. On the other side, EC and C, which have the same catechin skeleton as GCg, EGC, EGCg, and GC, showed effects similar to that of the control. GA showed moderate effect and retained 17.4% at 7 days (Table 1).

## DISCUSSION

$\beta$-Carotene, a kind of provitamin A, shows strong biological activity and is physiologically an important compound. $\beta$-Carotene contributes to human health

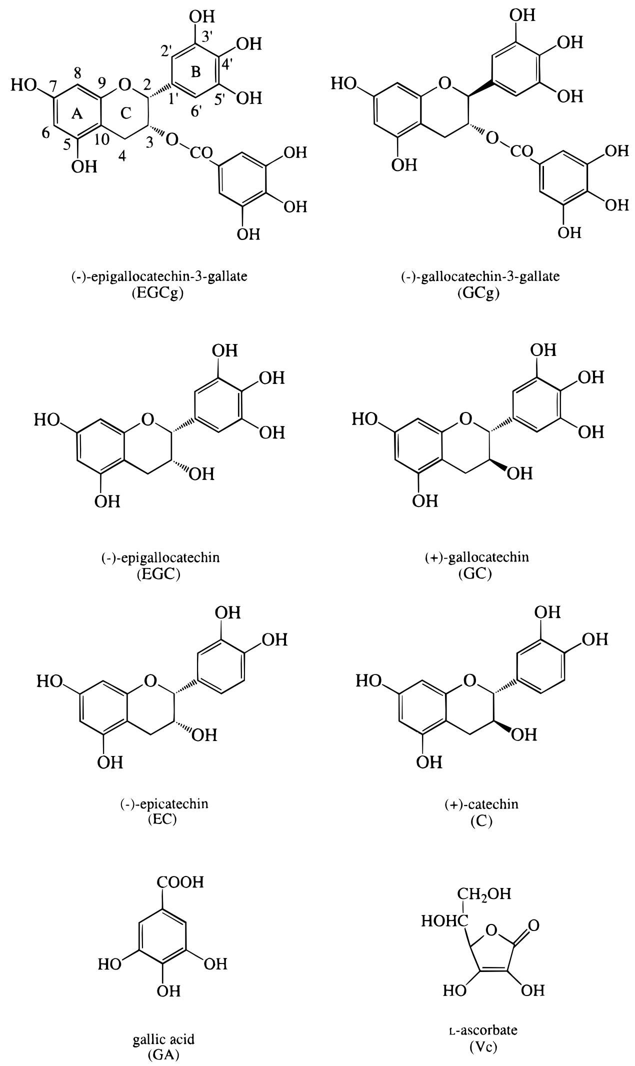


**Figure 5.** Structures of tea catechins, gallic acid, and L-ascorbate.

**Table 1. Antidiscoloring Effects of Catechins, Gallic Acid, and Ascorbate on β-Carotene**

| | residual ratio of β-carotene (%) | | |
|---|---|---|---|
| compd[a] (50 μM) | 5 days of incubation | 7 days of incubation | 10 days of incubation |
| EGCg | 83.5 | 77.8 | 33.0 |
| GCg | 91.0 | 83.8 | 57.4 |
| EGC | 87.6 | 69.9 | 48.1 |
| GC | 89.2 | 76.9 | 32.6 |
| EC | 80.4 | 4.0 | 1.1 |
| C | 87.8 | 2.5 | 0.2 |
| GA | 81.1 | 17.4 | 1.3 |
| Vc | 88.2 | 5.4 | 1.3 |
| control | 83.1 | 3.1 | 1.8 |

[a] EGCg, (−)-epigallocatechin 3-gallate; GCg, (−)-gallocatechin 3-gallate; EGC, (−)-epigallocatechin; GC, (+)-gallocatechin; EC, (−)-epicatechin; C, (+)-catechin; GA, gallic acid; Vc, L-ascorbate.

benefits because of its biological functions such as an anticancer effect. If β-carotene decomposed before its intake, biological functions in the body would not be observed. Furthermore, when β-carotene is used as a coloring agent for food products, the discoloration of its reddish color markedly reduces the worthiness of those products. In the present study, it was confirmed that tea polyphenols and several tea catechins have stronger effects against the discoloration of β-carotene than L-ascorbate, which is widely used to prevent the discoloration. Tea polyphenols showed a suppressive effect of degradation of β-carotene on both a water-soluble system using beverage and an oily system using margarine (Figures 1 and 2).

The effect of EGCg and Vc was compared with respect to concentration, showing about a 20-fold enhancement (Figure 3). With time, EGCg delayed the antidiscoloring effect to a better extent in terms of degradation of β-carotene (Figure 4). These observations can be probably due to the difference in the structures of EGCg and Vc, respectively.

It could be thought that the discoloration of β-carotene and the oxidation of unsaturated fatty acids progress by the same mechanism. That is, double bonds in carotene and unsaturated fatty acids are attacked by radicals, becoming peroxides. Antioxidants suppress the degradation of double bonds as radical scavengers. On the basis of structural observation, Hirose et al. discussed the mechanism of antioxidative activity of catechins. The hydrogen radical from the phenolic hydroxyl group of catechin stabilizes the peroxide radical formed during the oxidation process (Hirose et al., 1990a,b, 1991, 1995) . In the present study, it would be speculated that tea polyphenols delayed the degradation of β-carotene by acting as antioxidants following the same mechanism as described above. The results of the antioxidative effect of individual catechins, GA, and Vc were compared. EGCg, GCg, EGC, and GC showed strong discoloring activity, while EC, C, and Vc showed almost no activity, and GA had moderate activity. From these results, the structure−activity relationship was considered. When the effects of EGCg, GCg, EGC, and GC and of EC and C were compared, the hydroxy group at the 5′-position of the B-ring of the catechin structure was found to be an important contributing factor. Comparison between the effects of EGCg and GCg and of EGC and GC showed that the introduction of a galloyl group to the catechin skeleton did not enhance the activity. The following pairs, EGCg and GCg, EGC and GC, and EC and C, which are optical isomers each other, did not exhibit any difference in their discoloring activities. Therefore, the constitutional isomer of catechins did not enhance the activity. As GA showed stronger activity than Vc and the control, it was shown that the phenolic hydroxyl group acts as the hydrogen radical donor. The moderate activity of GA showed that not only the three phenolic hydroxyl groups but also the catechin skeleton is important for the activity. The results of these considerations suggest that for the antidiscoloring activity, the hydroxyl group at the 5′-position of the B-ring of the catechin skeleton is the most important factor to be considered.

Tea polyphenols and catechins, which have stronger activity than L-ascorbate, might be used as excellent antidiscoloring compounds of β-carotene.

ACKNOWLEDGMENT

We thank Dr. Bharati Ramchandra Joglekar for critically reading the manuscript.

LITERATURE CITED

Amarowicz, R.; Shahidi, F. Antioxidant activity of green tea catechins in β-carotene-linoleate model system. *J. Food Lipids* **1995**, *2*, 47−56.

Appel, M. J.; Woutersen, R. A. Effects of dietary β-carotene and selenium on initiation and promotion of pancreatic carcinogenesis in azaserine-treated rats. *Carcinogenesis* **1996**, *17*, 1411−1416.

Baranowitz, S. A.; Starrett, B.; Brookner, A. R. Carotene deficiency in HIV patients. *AIDS* **1996**, *10*, 115.

Chen, H.; Tappel, A. L. Protection of vitamin E, selenium, trolox C, ascorbic acid palmitate, acetylcysteine, coenzyme Q(0), coenzyme Q(10), Beta-carotene, canthaxanthin, and (+)-catechin against oxidative damage to rat blood and tissues in vivo. *Free Radical Biol. Med.* **1995**, *18*, 949−953.

Hirose, M.; Hoshiya, T.; Akagi, K.; Futakuchi, M.; Ito, N. Inhibition of mammary gland carcinogenesis by green tea catechins and other naturally occurring antioxidants in female Sprague-Dawley rats pretreated with 7,12-dimethylbenz[*a*]anthracene. *Cancer Lett.* **1994**, *83*, 149−156.

Hirose, Y.; Yamaoka, H.; Nakayama, M. Oxidation product of (+)-catechin from lipid peroxidation. *Agric. Biol. Chem.* **1990a**, *54*, 567−569.

Hirose, Y.; Yamaoka, H.; Nakayama, M. Oxidation product of (−)-epicatechin under radical reaction. *J. Jpn. Oil Chem. Soc.* **1990b**, *39*, 967−969.

Hirose, Y.; Yamaoka, H.; Nakayama, M. A novel quasi-dimeric oxidation product of (+)-catechin from lipid peroxidation. *J. Am. Oil Chem. Soc.* **1991**, *68*, 131−132.

Hirose, Y.; Fujita, T.; Shima, S.; Nakayama, M. An oxidative dimeric product of (+)-catechin by radical reaction in the dark. *J. Jpn. Oil Chem. Soc.* **1995**, *44*, 491−497.

Kajimoto, G.; Ikeda, Y.; Mukai, K. On the antioxidative components and antiseptic components in tea. Part IV. Antioxidative actions of materials extracted with various organic solvent from tea-grounds. *Eiyo To Syokuryo* **1969**, *22*, 473−479 (in Japanese).

Kohlmeier, L.; Hastings, S. B. Epidemiologic evidence of a role of carotenoids in cardiovascular disease prevention. *Am. J. Clin. Nutr.* **1995**, *62*, S1370−S1376.

Koketsu, M.; Satoh, Y. Antioxidative activity of green tea polyphenols in edible oils. *J. Food Lipids* **1997**, *4*, 1−9.

Lea, C. H.; Swoboda, A. T. The antioxidant action of some polyphenolic constituents of tea. *Chem. Ind.* **1957**, 1073−1074.

Matsuzaki, T.; Hara, Y. Antioxidative activity of tea leaf catechins. *Nippon Nôgeikagaku Kaishi* **1985**, *59*, 129−134 (in Japanese).

Moriguchi, S.; Okishima, N.; Sumida, S.; Okamura, K.; Doi, T.; Kishino, Y. *β*-Carotene supplementation enhances lymphocyte proliferation with mitogens in human peripheral blood lymphocytes. *Nutr. Res.* **1996**, *16*, 211−218.

Sakanaka, S.; Mujo, K.; Taniguchi, M.; Yamamoto, T. Antibacterial substances in Japanese green tea extract against *Streptococcus mutans*, a cariogenic bacterium. *Agric. Biol. Chem.* **1989**, *53*, 2307−2311.

Sakanaka, S.; Aizawa, M.; Mujo, K.; Yamamoto, T. Inhibitory effects of green tea polyphenols on growth and cellular adherence of an oral bacterium, *Porphyromonas gingivalis*. *Biosci., Biotechnol., Biochem.* **1996**, *60*, 745−749.

Sarkar, A.; Bishayee, A.; Chatterjee, M. Beta-carotene prevents lipid peroxidation and red blood cell membrane protein damage in experimental hepato carcinogenesis. *Cancer Biochem. Biophys.* **1995**, *15*, 111−125.

Yen, G. C.; Chen, H. Y. Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J. Agric. Food Chem.* **1995**, *43*, 27−32.

Zhi, Y. W.; Mou-Tuan, H.; You-Rong, L.; Xie, J.-G.; Kenneth, R. R.; Harold, L. N.; Chi-Tang, H.; Chung, S. Y.; Conney, A. H. Inhibitory effects of black tea, green tea, decaffeinated black tea, and decafeinated green tea on ultraviolet B light-induced skin carcinogenesis in 7,12-dimethybenz[*a*]anthracene-initiated SKH-1 mice. *Cancer Res.* **1994**, *54*, 3428−3435.

Ziegler, R. G.; Colavito, E. A.; Hartge, P.; Mcadams, M. J.; Schoenberg, J. B.; Mason, T. J.; Fraumeni, J. F. Importance of α-carotene, *β*-carotene, and other phytochemicals in the etiology of lung cancer. *J. Natl. Cancer Inst.* **1996**, *88*, 612−615.

Received for review September 30, 1996. Revised manuscript received February 28, 1997. Accepted March 3, 1997.[⊗]

JF9607526

[⊗] Abstract published in *Advance ACS Abstracts*, May 1, 1997.