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A study on the change of enantiomeric purity of catechins in green tea infusion

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Abstract

The enantiomeric composition of catechin in commercial tea beverages differs markedly from that in freshly brewed tea. We attempt to determine the cause of this difference by measuring the change in the ratios of catechins relative to (+)-catechin with time. When tea extracted with water was heated to 80 °C, (−)-epicatechin and (−)-epicatechin gallate epimerized to (−)-catechin and (−)-catechin gallate, respectively. When the thermal sterilized tea extracts were left at room temperature in the dark, it was recognized that the hydrolysis of the gallate moiety proceeded in parallel with epimerization. The hydrolysis of (−)-catechin gallate to (−)-catechin was a major pathway in the tea extracts that were not subjected to heat-treatment. Consequently, it appears that the simultaneous progression of the epimerization during thermal sterilization and the hydrolysis during distribution and storage increases the proportion of (−)-catechin ratio in the commercial tea beverages.

Keywords: Enantiomeric composition; Green tea; Catechins; Epimerization; Hydrolysis

1. Introduction

Recently, the effects of food ingredients on human health have been reconsidered. Tea is one of the most widely consumed beverages in the world, and it is recognized for its high content of polyphenols, in particular tea catechins. Catechins have received a great deal of attention due to their many biological activities (Arts, Hollman, Feskens, Mesquita, & Kromhout, 2001; Chen, Yang, Jiao, & Zhao, 2002; Mabe, Yamada, Oguni, & Takahashi, 1999; Maeda-Yamamoto, Kawahara, Tahara, Tsuji, Hara, & Isemura, 1999; Riemersma, Rice-Evans, Tyrrell, Clifford, & Lean, 2001; Unno, Yayabe, Hayakawa, & Tsuge, 2002; Zhong et al., 2002), such as antioxidative activities (Cao, Sofic, & Prior, 1996; Deng, Tao, Li, He, & Chen, 1998; Dulloo et al., 1999; Leung, Su, Chen, Zhang, Huang, & Chen, 2001; Pietta, Simonetti, & Mauri, 1998; Shu-Wen & Edwin, 1997) and antitumour activities (Constable, Varga, Richoz, & Stadler, 1996; Weyant, Carothers, Dannenberg, & Bertagnolli, 2001). There are many related compounds in catechins, and they include the stereoisomers resulting from two asymmetric carbons. Catechins seem to exist in one enantiomeric form in tea leaves, e.g. catechin (CA; 2-[-3,4-dihydroxyphenyl]-3,4-dihydro-1-[2H]-benzopyran-3,5,7-triol) is the (+)-isomer of the (2R, 3S)-configuration. However, the compositions of catechins in tea extract change during heat-processing and storage (Stach & Schmitz, 2001; Wang & Helliwell, 2000; Wang, Kim, & Lee, 2000; Zhu, Zhang, Tsang, Huang, & Chen, 1997). The dominant configuration of the enantiomeric composition of CA in commercial tea beverages is the (−)-isomer of (2S, 3R)-configuration (Yang, Arai, & Kusu, 2000). Catechins undergo epimerization at the C-2 position in hot (Seto, Nakamura, Nanjo, & Hara, 1997) and/or high pH solution (Chen, Zhu, Tsang, & Huang, 2001; Mehta, & Whalley, 1963; Suematsu, Hisanobu, Saigo, Matsuda, Hara, & Komatsu, 1993), and this reaction may have a role in the change in composition and/or optical purity of catechins.

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Although a large number of studies have examined the physiological functions of catechins, little is known about the differences in biological activities between their enantiomers. The physiological effects of commercial tea beverages may differ from those of brewed teas. The aim of this present study is to clarify the changes in enantiomeric composition of CA and epicatechin (EC) in green tea extracts during heat-processing and storage. We also propose a mechanism that can account for such changes.

2. Materials and methods

2.1. Samples

Green tea leaf ('yabukita', Shizuoka, Japan) and commercial tea beverages, in polyethylene terephthalate (PET) bottles were purchased from several local markets in Tokyo.

2.2. Standards

Tea catechins, (+)-CA, (−)-CA, *rac*-CA, (+)-EC, (−)-EC, (−)-epicatechin gallate [(−)-ECG], and (−)-catechin gallate [(−)-CG] were obtained from Sigma Aldrich Japan (Tokyo, Japan). HPLC-grade acetonitrile, ethanol and *n*-hexane were purchased from Wako Pure Chemical Industries (Osaka, Japan). Formic and acetic acids from Wako were of guaranteed grade. Water was purified with a Milli-Q gradient A10 Elix system (Millipore, Bedford, MA, USA).

2.3. HPLC apparatus and conditions

The LC system consisted of a Shimadzu (Kyoto, Japan) LC-10ADvp pump, a Rheodyne (Cotati, CA, USA) 7725i injector with a 20-μl loop, a Shimadzu SPD-M10Avp photodiode-array detector (DAD) and a Shimadzu CTO-10Avp column oven. Chromatographic separation of catechins was performed on a 250×4.6 mm i.d. reversed-phase column (CAPCALL PAK C_{18} UG120; Shiseido, Tokyo, Japan) maintained at 40 °C. A mobile phase, consisting of water/acetonitrile/formic acid (91.5/8/0.5; v/v), was delivered at a flow rate of 1 ml/min. Enantiomeric separations of CA and EC were performed on a Chiralcel OC column (250×4.6 mm i.d.; Daicel Chemical Ind., Osaka, Japan), maintained at 40 °C. A mobile phase, consisting of *n*-hexane/ethanol/acetic acid (64.5/35/0.5; v/v), was delivered at a flow rate of 1 ml/min. The enantiomers were monitored using a fluorescence detector (Shimadzu RF-10A_{XL}) at excitation: 280 nm and emission: 315 nm.

2.4. Data processing

All of the acquisition data were converted into spreadsheet software (Excel, Microsoft Co., Redmond, WA, USA), and then smoothed by the method of Savitzky and Golay (1964) to calculate the peak areas with accuracy. The calculation of total CA on the reversed-phase column was performed by using our derivative spectrum chromatogram method (Yamamoto, Matsunaga, Ohto, Mizukami, Hayakawa, & Miyazaki, 1995).

2.5. Sample preparation

Five grammes of tea leaves were extracted with 100 ml of water or water/acetonitrile (1/1; v/v) at room temperature using an ultrasonic bath for 30 min. To examine the change of catechins with heating time, samples of tea extract were put into glass tubes and heat-processed at 80 °C for various periods. To determine changes with long-term storage, tea extracts that had been heat-processed for 5 min were stored in the dark at room temperature for up to 70 days. Samples of tea extracts prepared with water/acetonitrile were stored for up to 28 days without thermal sterilization. The sample solutions were filtered through an acrodisc 0.45-μm syringe filter, and then applied to the reversed-phase HPLC. The CA and EC fractions from the reversed-phase column were collected at the same time. After evaporating to dryness under nitrogen, the residue was reconstituted in ethanol. Aliquots of these solutions were injected into the chiral HPLC system.

3. Results and discussion

3.1. Optical purity of CA and EC in commercial PET-bottled tea beverages

Concentration and optical purity of CA and EC in three commercial PET-bottled tea samples and a brewed tea sample were determined. There were many impurities in the freshly brewed tea, so the achievable resolution of CA and the impurities was not sufficient to quantify small amounts of CA on the reversed-phase column. The advent of DAD for HPLC has increased the potential for the quantification of the overlapped peaks. The derivative spectrum chromatography (Yamamoto et al., 1995) is one of the multivariate resolution methods using DAD. In this experiment, the second-derivative spectrum chromatogram at 280 nm was successful for the quantification of CA with high precision. On the other hand, the resolutions (*Rs*) between enantiomers of CA and EC were 2.04 and 1.49 on the chiral column, respectively. The enantiomeric compositions of CA in the PET-bottled tea beverages and brewed tea differed markedly (Table 1). The C ring of the flavanol skeleton is opened at C-2, corresponding to a benzyl position, by high-temperature heat-processing of tea beverages. When the ring closes, the C-2

Table 1
Enantiomeric compositions of catechin and epicatechin in PET-bottled and brewed tea drinks

	PET-bottled green tea			Freshly brewed tea
	(A)	(B)	(C)	
(+)-catechin (mg/ml)	0.008	0.005	0.006	0.052
(−)-catechin (mg/ml)	0.059	0.049	0.034	0.006
(+)-epicatechin (mg/ml)	0.002	0.001	0.041	0.000
(−)-epicatechin (mg/ml)	0.043	0.033	0.041	0.661
(+)-epicatechin/(+)-catechin	0.21	0.24	0.29	0.00
(−)-epicatechin/(−)-catechin	0.73	0.73	1.20	118

position is sometimes inverted. CA is thermodynamically more stable than EC. This is because the 2-gallyl and 3-hydroxy groups in CA are *trans*-related while in EC they are *cis*-related. Epimerization of (−)-EC to (−)-CA is reported to be 2–3 times more rapid than that of (−)-CA to (−)-EC (Kiatgrajai, Wellons, Lawrence, & White, 1982). The epimerization between CA and EC results in an equilibrium mixture in which CA predominates over EC and at equilibrium, the ratios of (−)-EC to (−)-CA and (+)-EC to (+)-CA should become equal. As shown in Table 1, however, the values of (−)-EC/(−)-CA in commercial PET-bottled tea beverages were significantly larger than those of (+)-EC/(+)-CA. It should be noted that tea leaves contain a large amount of (−)-ECG, so the possibility that the hydrolysis of (−)-ECG generates (−)-EC can not be ruled out. Similarly, the generation of (−)-CA by the hydrolysis from (−)-CG, which is generated by the epimerization of (−)-ECG, is also possible (Fig. 1). The presence of these hydrolysis products was confirmed in the following experiments.

3.2. Changes in catechins of green tea extract during heat processing

Catechins also undergo oxidation and/or polymerization under high temperature and high pH conditions, and their concentrations decrease rapidly (Komatsu, Suematsu, Hisanobu, Saigo, Matsuda, & Hara, 1993). In order to understand the effect of thermal epimerization on the composition of catechins, the tea extracts were heat-treated at the relatively low temperature of 80 °C. Moreover, to evaluate the results of measurement precisely, catechin concentrations were expressed relative to the concentration of (+)-CA because CA has no hydrolyzing gallyl moiety and is considered to be scarcely epimerized to (+)-EC. Hardly any (+)-EC was generated during heat-processing at 80 °C. Fig. 2 shows the influence of heating time of the tea extract on the change in concentrations of catechins. In freshly brewed tea, (+)-CA predominated over (−)-CA and (−)-EC

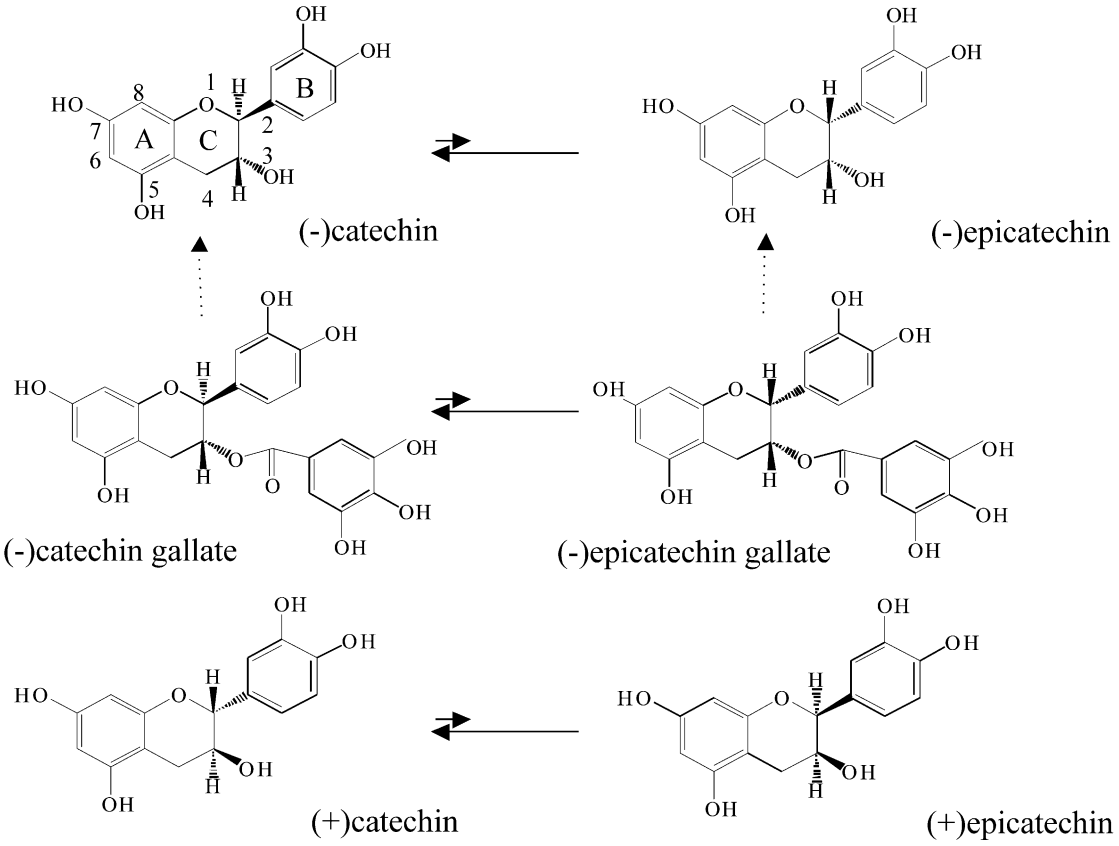

Fig. 1. Structures of catechins determined in this work and their correlation. Solid arrows indicate epimerization and dotted ones are hydrolysis.

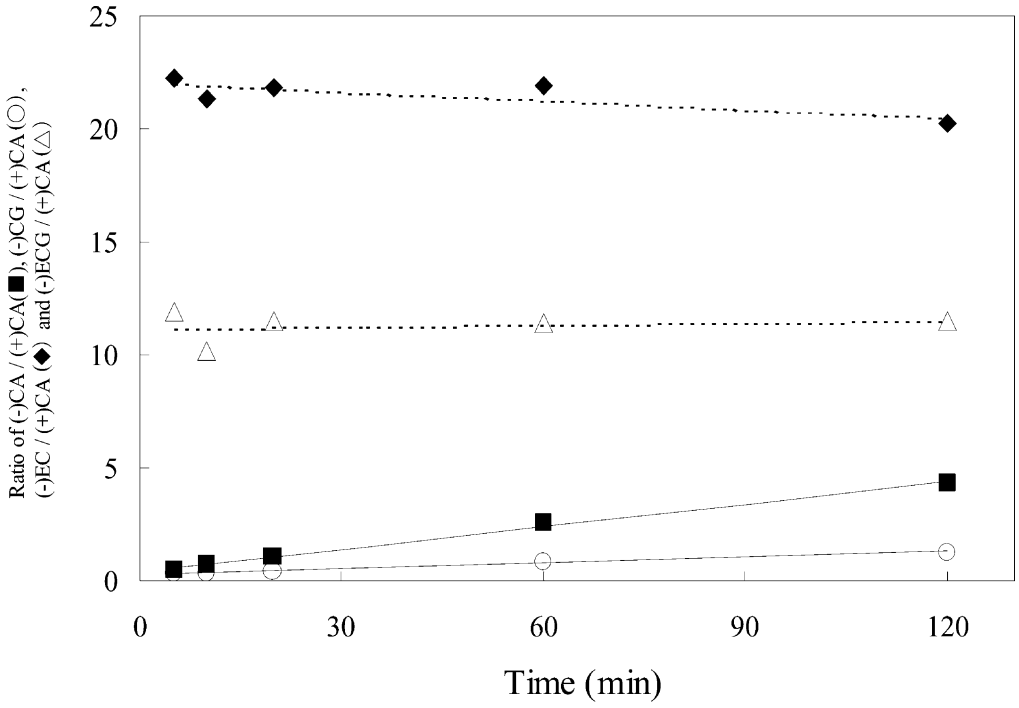

Fig. 2. Effect of heating time on the relative concentrations of catechins. Each plotted point is the mean of duplicate or triplicate sample analyses.

was the only EC enantiomer. Although the proportion of (−)-CA increased with heating time, that the proportion of (−)-EC decreased. The sum of the relative quantities of (−)-EC and (−)-CA was almost constant during heat processing, and suggesting that (−)-EC quantitatively epimerized to thermodynamically stable (−)-CA. While the apparent quantity of (−)-CG slightly increased with heating time, there is no clear change in quantity of (−)-ECG. Although (−)-ECG is more thermodynamically unstable than the other three catechins, the ratio of (−)-ECG to (+)-CA did not decrease during heat-processing. Under this thermal condition, the oxidative degradation of (+)-CA may be more rapid than that of (−)-ECG.

3.3. Changes in catechins of green tea extract during storage

Sterilized tea extracts were left for 10 weeks in the dark at room temperature. The extracts took 2 weeks to remove the effect of heat-treatment. In this period, the concentrations of catechins changed irregularly and then stabilized. The results up to 70 days are shown in Fig. 3. While the relative quantity of (−)-CA increased linearly during the period from the 2nd week to the 10th week, that of (−)-EC remained almost constant. On the other hand, (−)-CG increased but (−)-ECG decreased. It should be noted that the sum of the relative quantities of these four catechins remained almost constant. This can be explained as follows: during storage at room temperature, the apparent quantity of (−)-ECG continuously decreased as a result of both the hydrolysis to (−)-EC and epimerization to (−)-CG. Although the apparent quantity of (−)-CG increased by epimerizing from (−)-ECG, (−)-CG would be expected to simultaneously hydrolyze to (−)-CA. Similarly, the relative quantity of (−)-EC is dependent on the difference in rates between the epimerization to (−)-CA and the hydrolysis from (−)-ECG. In the case of (−)-EC, the two rates were almost equal. Together, the epimerization from (−)-EC and the hydrolysis from (−)-ECG resulted in a linear increase in (−)-CA. We may, therefore, reasonably conclude that catechins in tea leaves' extracts simultaneously underwent epimerization and hydrolysis during storage at room temperature after heat-treatment.

3.4. Changes in catechins extracted from tea leaves without heat-treatment

To determine the changes occurring in extracts that are not subjected to heat-treatment, the extraction solvent was replaced with water/acetonitrile (1/1; v/v), and the extracts were left for 7 weeks at room temperature without thermal sterilization. Clear change in relative quantities of each catechin appeared immediately, as was expected. The results up to 49 days are shown in Fig. 4. The ratio of (−)-CA to (+)-CA tended to increase and that of (−)-CG tended to decrease. The increase in (−)-CA was clearly related to the decrease in (−)-CG. On the other hand, there were no clear tendencies of changes in (−)-ECG and (−)-CG, whose initial concentrations were relatively high. We can be

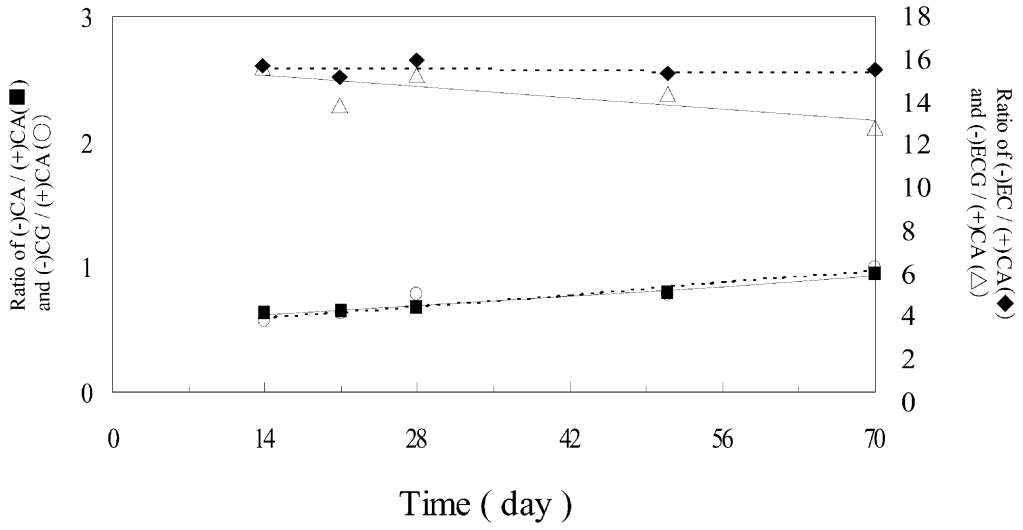

Fig. 3. Changes in the relative concentrations of catechins extracted from tea leaves with water during storage at room temperature after sterilization at 80 °C for 5 min. Each plotted point is the mean of duplicate or triplicate sample analyses.

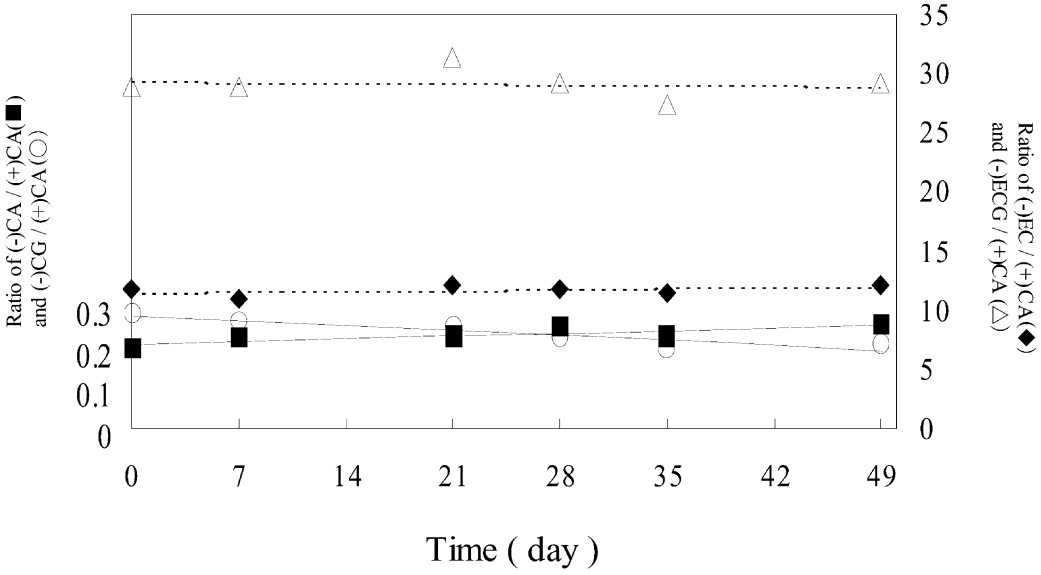

Fig. 4. Changes in the relative concentrations of catechins extracted from tea leaves with water-acetonitrile during storage at room temperature without thermal sterilization. Each plotted point is the mean of duplicate or triplicate sample analyses.

fairly certain that the catechins were subject to, not only epimerisation, but also hydrolysis of the gallyl moiety.

In conclusion, the change in optical purity of CA in a commercial tea beverage appears to be due to the combined effects of epimerization and hydrolysis during thermal sterilization and storage. Generally, there are different biological activities between enantiomers. The same may be said of catechins. To date, little has been clarified about the different functions of (+)-CA and (−)-CA, such as glycogen metabolism (Nyfeler, Moser, & Walter, 1983). However, it will become more clear before long. In the manufacture of tea beverage, it is important to control the changes in not only catechin composition but also the optical purities.

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