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Food Chemistry 67 (1999) 429–433

Food Chemistry

www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods Section

Efficiency of the extraction of catechins from green tea

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Received 15 February 1999; received in revised form and accepted 5 May 1999

Abstract

The effects of various experimental conditions that may affect the extraction efficiency of green tea catechins were studied using aqueous buffers. Some catechins were absorbed by certain types of filtration membranes which caused errors in their analysis. At higher pH conditions, the amounts of the major catechins (EC, EGC, ECg and EGCg) extracted from tea were decreased and the amounts of minor catechins (C, GC, Cg and GCg) were increased. This observed shift in the levels extracted is considered a consequence of epimerization of the major catechins to the minor catechins. In general, the major differences in the extraction efficiency of major catechins by difference of pH were observed with a lower tea concentration. Also, at the same pH value, extraction efficiencies varied, depending on the catechins and the tea–water ratio.

Keywords: Catechins; Green tea; Extraction; Filter

1. Introduction

Green teas are consumed in Japan and China as one of the most popular and traditional non-alcoholic beverages. Also, green teas are receiving considerable attention for specific health claims and because of the presence of functional constituents, such as caffeine, ascorbate and catechins. Among these constituents, caffeine is well known for its stimulant effect and is present at 2 to 4% of dried tea leaf weight, depending on the types and quality of teas (Goto, Yoshida, Amano & Horie, 1996a). Green tea catechins are present at 8 to 15% of dry leaf weight (Goto, Yoshida, Amano, et. al., 1996a).

The catechins are composed of a family of four major substances, epicatechin (EC), epicatechin gallate (ECg), epigallocatechin (EGC), epigallocatechin gallate (EGCg) and four minor catechins, catechin (C), catechin gallate (Cg), gallocatechin (GC) and gallocatechin gallate (GCg) as epimers of the major catechins. These catechins contribute to the characteristic bitter and astringent taste (Thorngate & Noble, 1995) of tea along with the brothy and sweet taste from amino acids such as theanine, glutamic acid and arginine (Nakagawa, 1975). Recently, catechins have received considerable attention both from the scientific community and the general public because of their claimed health benefits and functionality such as anti-oxigenicity (Matsuzaki & Hara, 1985; Wiseman, Balentine & Frei, 1997), anti-mutagenicity (Cheng et al., 1991), anti-tumorgenicity (Hagiwara et al., 1991; Hara, Matsuzaki & Nakamura, 1989) and anti-carcinogenicity (Chen, Schell, Ho & Chen, 1998).

Of course, the methodology and extraction efficiency of catechins from tea is critical in further studying the functionality of these substances. In general, organic solvents such as methanol and acetonitrile have been used as solvents to quantitatively extract catechins from tea leaves. Suematsu, Hisanobu, Saigo, Matsuda and Komatsu (1995) studied the extraction condition of catechins from tea leaves and proposed acetonitrile–water (1:1, v/v) as an efficient extraction solvent. This method is very useful for measuring catechins in leaves; however, for studying human consumption of tea, extraction using these organic solvents may not reflect actual levels of the catechins in the tea beverage. Some studies on the extraction condition of tea using hot water were reported. However, the purpose of these

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0308-8146/99/$ - see front matter
PII: S0308-8146(99)00148-X

studies was to investigate the best condition for sensory evaluation of tea quality by color, flavor and taste (Kubota, Takeo, Hara & Saito, 1959), or to survey the taste qualities of tea (Ikeda, Nakagawa & Iwasa, 1972) or to make most tasty teas (Research Group of Green Tea Brewing, 1973), and provided little quantitative information about the chemical constituents in the beverage.

In this study, we examine the effects of the pH of water and the tea–water ratio on the efficiency of the extraction of catechins as it may relate to tea consumption and other usages (Ui et al., 1991). In addition, the effects of the filter membrane on the recovery of individual catechins are reported.

2. Materials and methods

2.1. Sample and chemicals

Eight catechin standards, EC, ECg, EGC, EGCg, C, Cg, GC and GCg, were purchased from Kurita Kogyo Ltd. (Tokyo, Japan) and Polyphenon 60, partially purified green tea extract containing about 60% of catechins of dry weight, was donated from Mitsui Norin Co. Ltd. (Tokyo, Japan). Anhydrous caffeine was purchased from Wako Pure Chem. (Osaka, Japan) and all other chemicals were either GR grade or HPLC grade without further purification. Bower and Bates buffer (Perrin & Dempsey, 1974) was prepared using 0.2 M sodium hydroxide and 0.2 M potassium dihydrogen phosphate. The pH of the buffer and extracts were measured by an IOL-40 pH meter (DKK, Tokyo, Japan) with a glass electrode (6157–0.65 W, DKK).

A green tea sample was prepared at Tokyo Metropolitan Agricultural Experiment Station using the first crop of 'Yabukita' leaf in 1997. The sample was milled with a chemical mill (Nippon Rikagaku Kikai, Tokyo, Japan) and passed through a 1 mm mesh screen. The milled sample was stored at −30°C until used. The catechins in this sample were measured by the method of Goto, Yoshida, Kiso and Nagashima (1996b). The levels of catechins in this tea were EC, 9.90 mg/g; ECg, 12.4 mg/g; EGC, 40.6 mg/g; EGCg, 71.7 mg/g; C, 1.17 mg/g; Cg, 0.87 mg/g; GC, 2.81 mg/g; GCg, 1.72 mg/g of dry weight. Caffeine content of this tea was 25.5 mg/g dry weight.

Samples were extracted with 100 ml of either buffer or distilled water at 80°C with constant gentle shaking for 20 min. The extracts were filtered through a filter cartridge (DISMIC 13HP, Advantec Toyo, Tokyo, Japan) prior to HPLC analysis.

2.2. HPLC analysis

The levels of catechins and caffeine in the extracts were measured by the method previously developed (Goto, Yoshida, Kiso, et al., 1996b). The HPLC system consisted of Shimadzu LC-6AD pumps with a two pump gradient system (Shimadzu Co. Ltd., Kyoto, Japan), a Shimadzu SPD-6AV UV–VIS detector, a Shimadzu SCL-10AXL auto sample injector, and a Model 556 column oven (GL Science, Tokyo). The column was a Develosil ODS-HG column (150×4.6 mm, Nomura Chem. Co., Seto, Japan) equipped with a guard column (10×4 mm, Nomura). The flow-rate of the mobile phase was 1 ml/min.

The mobile phase compositions used were: (A) water–acetonitrile–85% phosphoric acid (95.45:4.5:0.05, v/v/v); (B) water–acetonitrile–85% phosphoric acid (49.95:50.0:0.05, v/v/v). The solvent composition started at 90% solvent A and 10% solvent B and was maintained for 5 min, then increased to 30% solvent B in 3 min. This condition was maintained for 2 min followed by an increase of solvent B to 80% in 5 min. The final conditions were held for an additional 5 min.

3. Results

3.1. Effect of filters on recovery

A mixture of catechins and caffeine dissolved in water was passed through various types of disposable cartridge filters that use different filtration membranes, and the recovery of each chemical was measured by HPLC. Recovery of caffeine and non-ester type catechins, EC and EGC, were quantitatively similar for all the different types of filters. However, the recovery for gallate catechins, ECg and EGCg, were different, depending on the type of filter. After samples were passed through cartridges which use polyvinyldifluoride or regenerated cellulose as membranes, the recovery of EGCg and ECg were decreased relative to the other membranes (Table 1). These results are similar to the results previously shown using acetonitrile containing solvent (Goto,

Table 1
Recovery of catechins and caffeine from filter cartridge[a]

Membrane material	EC	ECg	EGC	EGCg	Caffeine
PTFE[b]	100	97.8	99.8	99.2	99.7
PTFE	101	99.3	100	99.8	100
RC[c]	96.3	87.1	94.1	86.5	98.9
PVDF[d]	97.3	8.6	96.5	30.2	99.2
PVDF	99.9	63.0	99.1	79.1	99.9
CA[e]	100	92.6	99.2	96.6	99.7

[a] One ml of polyphenone 60 solution (0.33 mg/ml of water) was passed through filters and measured by HPLC.
[b] Polytetraflouroethylene with a hydrophilic coating.
[c] Regenerated cellulose.
[d] Polyvinyldifluoride.
[e] Cellulose acetate.

Yoshida, Kiso, et al., 1996). Therefore we decided to use hydrophilic PTFE filters for sample preparation in this study.

3.2. Epimerization of catechins during extraction

A 1% suspension of green tea was extracted under three different pH conditions, pH 6, 7 and 8, and the levels of extracted catechins and caffeine were compared to that extracted using a 50% acetonitrile solution. In a 50% acetonitrile extract, the level of caffeine was practically the same as the levels using the three different pH solutions. In the buffered solutions, the amounts of the various catechins were different from the levels detected in the 50% acetonitrile extract. However, the total amounts of the eight catechins amongst the buffer solutions were not different, and were about 70% of the levels measured in the 50% acetonitrile extract.

The levels of individual catechins in each buffer were consistently different. When the pH of extractant was increased, the levels of the major catechins, EC, EGC, ECg and EGCg were decreased (Fig. 1). In contrast, the levels of the minor catechins were dramatically increased with an increase of pH. However, there were small differences of the total amounts of the paired epimer catechins, GC + EGC, C + EC, GCg + EGCg and Cg + ECg among these three extracts.

Similar patterns were observed using catechin standards and buffers. Catechins were dissolved in different pH buffers and the solutions maintained for 20 min at 80°C, followed by measurements of the individual catechins. Under these conditions, the amount of EGC in buffers at pH 6, 7 and 8 decreased by 71, 37 and 12%, respectively. Interestingly, the amount of GC, the epimer of EGC, increased by 102, 168 and 199%, respectively. Similar results were obtained for the other three sets of catechins (Table 2). These results suggest that the major catechins epimerize to the minor catechins during extraction at higher pH.

3.3. Effects of tea-water ratio for catechins extraction

Green tea catechins were extracted with distilled water at five different tea–water ratios, 0.25, 0.5, 1.0, 2.0 and 3.0%, at 80°C for 20 min. The resultant changes in the pH of the extract are shown in Fig. 2. The pH of water before extraction was almost neutral (pH 7.2) and, with

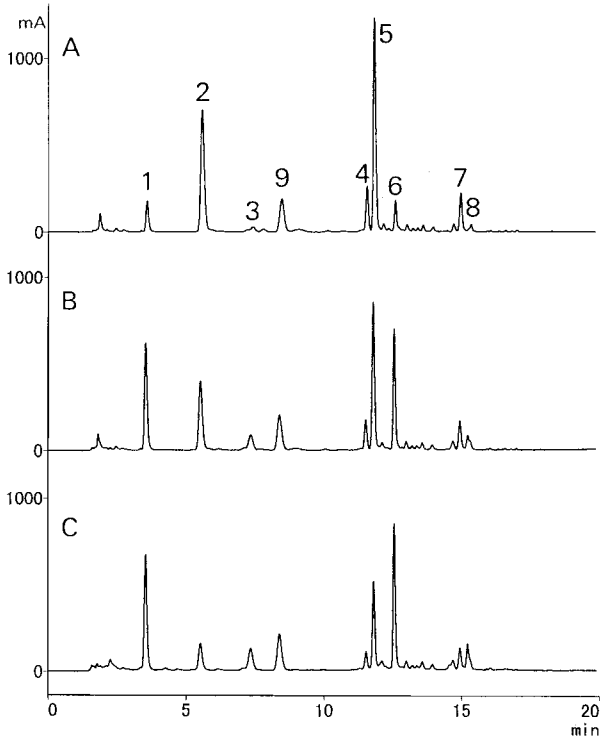

Fig. 1. Chromatograms of green tea extract by different pH of extractant A: extract by buffer at pH 6; B: extract by buffer at pH 7; C: extract by buffer at pH 8. Peaks: 1, gallocatechin; 2, epigallocatechin; 3, catechin; 4, epicatechin; 5, epigallocatechin gallate; 6, gallocatechin gallate; 7, epicatechin gallate; 8, catechin gallate; 9, caffeine.

Table 2
Changes (%) of catechins in different pH solutions[a]

	EC	ECg	EGC	EGCg
pH 6	98.2	95.5	70.9	91.6
pH 7	66.6	68.4	36.9	57.9
pH 8	61.0	52.6	11.8	22.8
	C	Cg	GC	GCg
pH 6	106	124	102	142
pH 7	266	286	168	509
pH 8	318	307	199	509

[a] Polyphenone 60 (0.33 mg/ml) were dissolved in different buffers and the solutions maintained at 80°C for 20 min. After 20 min of incubation amounts of individual catechins were compared to those of dissolved in 50% acetonitrile–water.

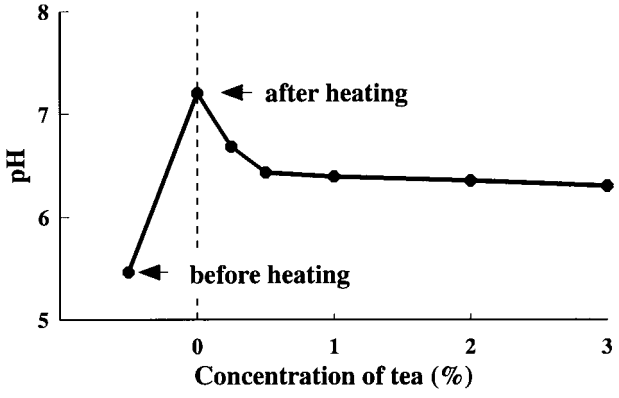

Fig. 2. Changes of pH of the extract by tea concentration. Green tea was extracted at concentrations from 0.25 to 3% using distilled water. After the extracts were cooled to room temperature, the pH values of the extracts were measured.

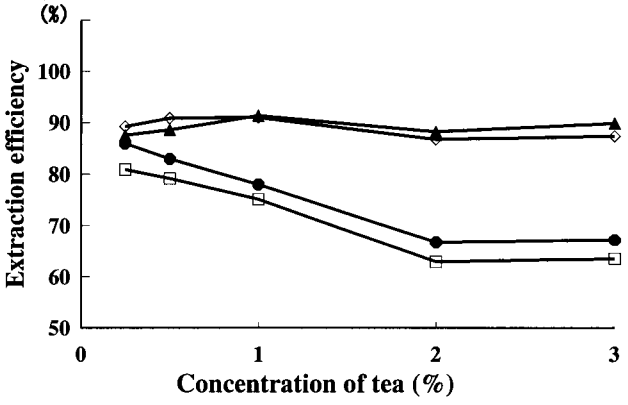

Fig. 3. Effect of tea concentration on the extraction efficiency. Green tea was extracted at tea concentrations from 0.25 to 3% by distilled water. ◇, epigallocatechin; ▲, epicatechin; □, epigallocatechin gallate; ●, epicatechin gallate.

extraction, the pH decreased to 6.7 at 0.25% and 6.4 at 0.5%. As the tea concentration increased up to 3%, only a very slight decrease of pH was observed.

The extraction efficiencies of the four major catechins at different tea concentrations are shown in Figs. 2 and 3. Varying the tea concentration did not affect the high extraction efficiency of the free catechins, EC and EGC. The extraction efficiency for the gallate catechins, ECg and EGCg, were lower than those of free catechins and decreased with increasing tea concentrations. However, a corresponding increase of minor catechins with a concomitant decrease of major catechins, when observed with changes in the pH, were not observed with changes in tea concentration. So these changes of extraction efficiency of gallate catechins were likely not the result of epimerization of these catechins.

3.4. Effects of pH and tea concentration on catechins extraction

As previously shown, the major catechins epimerize to minor catechins (Fig. 1) with increasing pH of the extractant. Also, the tea–extractant ratio affects both the pH of the extract and the efficiency of catechin extraction (Fig. 3). To demonstrate the effects of both pH of the extractant and tea concentration on extraction efficiency, tea was extracted with buffers of 14 different pHs, pH 6.0 and pH 6.4 to pH 7.6 at pH 0.1 intervals, at tea–extractant ratios of 0.25, 0.5, 1.0 and 3.0% (Fig. 4).

In general, at higher pH conditions, the extraction efficiency of the major catechins decreased, with greater differences being observed at lower tea concentration. At the same tea concentrations, the differences in the extraction efficiencies of non-gallate catechins were greater than those of gallate catechins with increasing pH. In comparing the gallo catechins, EGC and EGCg, to the non-gallo catechins, EC and ECg, larger changes of extraction efficiencies due to the pH of the extractant and the tea concentration were observed in gallo catechins. The difference of extraction efficiencies of EGC due to tea concentration below pH 6.7 were less than

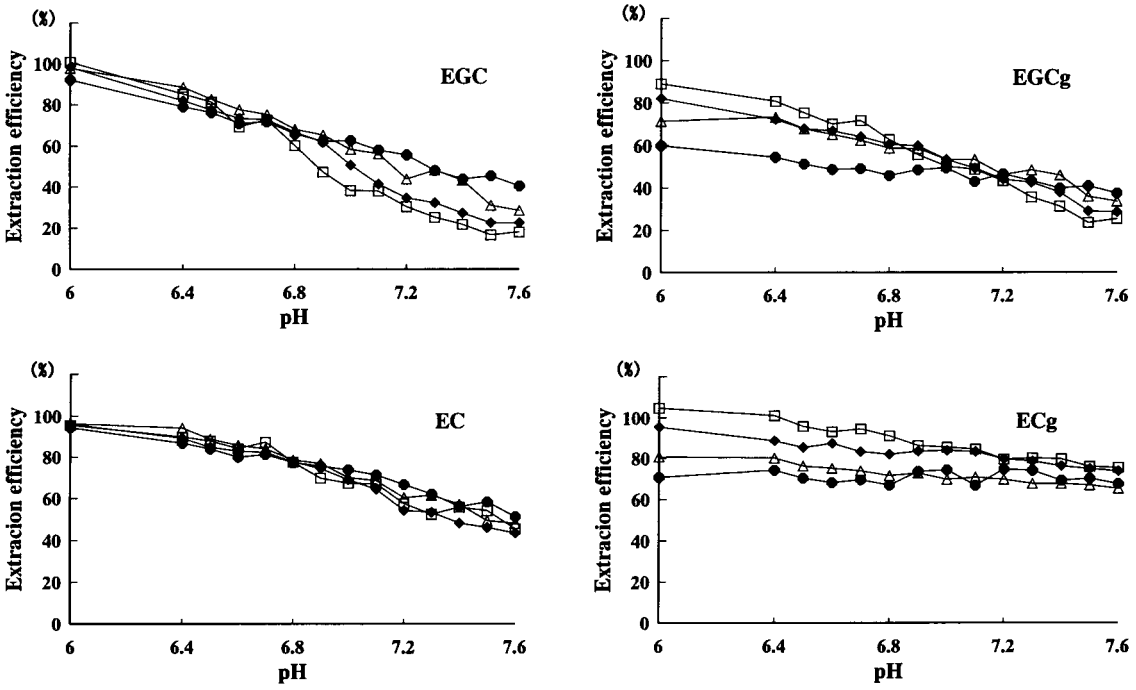

Fig. 4. Effects of pH of extractant and tea–extractant ratio on the extraction efficiency of green tea catechins. Green tea was extracted by Bower and Bates buffers. Tea/extractant ratio (g/w) (□, 0.25%; ◆, 0.5%; △, 1.0%; ●, 3.0%).

10%, and the overall extraction efficiencies were more than 70%. However, as the pH increased to 7.6, the extraction efficiencies decreased. Likewise, as the tea concentration decreased from 3 to 0.25%, the extraction efficiencies decreased from 40% to 18%, respectively. For EGCg, the effects of changes in the tea concentration were variable, depending on the pH. At a pH of less than 7.0, the extraction efficiencies at lower tea concentration were greater than that of higher tea concentrations. But, once the pH of the extractant became more than 7.2, the extraction efficiencies at higher tea concentrations became greater than that of the lower tea concentrations (Fig. 4).

The differences of tea concentrations throughout these pH ranges did not affect the extraction efficiencies of EC. The efficiency dropped from more than 90% at pH 6.0 to 44–51% at pH 7.6.

The extraction efficiencies of ECg at higher tea concentrations were not significantly affected throughout these pH ranges. However, the efficiencies of lower tea concentrations slightly decreased with the increase of pH of the extractant. The differences of the extraction efficiency, depending on the tea concentration, decreased from 30% at pH 6.0 to 15% at pH 7.6.

4. Discussion

Only small differences of the extraction efficiencies of EGC and EC were observed with changes in tea concentration (Fig. 3). This result was similar to the results using buffer solutions between pH 6.4 to 6.7 as extractant (Fig. 4). The concentration of the tea had a greater impact on the extraction efficiency of gallate catechins compared to changes in the pH (Figs. 2 and 3).

These experiments clearly show that both pH of the extract and tea concentration may affect the extraction efficiency of green tea catechins. In general, organic solvents, such as methanol and acetonitrile, efficiently extracted green tea catechins without epimerization of the major catechins (Suematsu et al., 1995). But, for situations where organic solvents are not acceptable, extraction at lower pH can also prevent epimerization of major catechins. However, below pH 7, the extraction efficiencies of gallate catechins decreased with an increase in the tea concentration (Fig. 4). Therefore, depending on the purpose and requirements, the concentration of tea must be considered carefully for effective extraction of the catechins.

Acknowledgement

The authors thank Dr. Mark Y. Fukayama for assistance with this manuscript.

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