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Catechin stability of EGC- and EGCG-enriched tea drinks produced by a two-step extraction procedure

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ABSTRACT

Catechin content of green tea drinks commercially available is reported to be very low in comparison with tea traditionally prepared, due to catechins conversion to their corresponding epimers during production. The purpose of this present study was to produce catechin-enriched tea drinks according to a two-step brewing procedure and to verify the catechin stability of those enriched drinks during storage. Those results confirmed that it is possible to produce EGC- and EGCG-enriched tea drinks regardless of the green tea used. Good extraction efficiencies were reached for the first and second extraction steps with catechin extraction yields ranging from 63.6% to 84.8%. Furthermore, it appeared that the catechin content in the two enriched tea drinks demonstrated a great stability since no significant degradation occurred within 8 weeks of storage. This simple two-step extraction procedure could be considered as an interesting way to produce enriched green tea drinks with more potent and stable bioactive catechins.

1. Introduction

Among different types of teas, green tea (a non-fermented tea) is of particular interest due to health benefits associated with its high catechin content. These beneficial effects range from antioxidant, anticarcinogenic, antimutagenic, anti-inflammatory to antimicrobial activities (Fujiki, 1999). Consequently, on the nutraceutical and health market, canned and bottled green tea drinks are getting more and more popular worldwide (Mukhtar & Ahmad, 2000). However, Chen, Zhu, Tsang, and Huang (2001) demonstrated that the catechin content of green tea or assimilated green tea drinks commercially available was very low in comparison with tea traditionally prepared. These authors concluded that the catechins have been converted to their corresponding epimers during their production/manufacturing.

The stability of green tea catechins in commercial drinks is of major concern (Su, Leung, Huang, & Chen, 2003), but stability of green tea catechins in tea drinks has not received much attention. To the best of our knowledge, only two studies have been published on green tea catechin stability in solutions and drinks. Su et al. (2003) observed that green tea catechin were vulnerable to degradation caused by elevation of temperature and pH of incubation media. However, for their long-term stability study in different solution and commercial soft drinks, Su et al. (2003) used green tea catechins obtained by extraction with chloroform to remove caffeine and pigments, and thereafter with ethyl acetate. In addition, they only tested this green tea catechin extract in combination with a theaflavin extract. In their contribution Chen et al. (2001) observed that, in commercially soft drinks, catechins showed varying stability with (−)-epigallocatechin gallate (EGCG) and (−)-epigallocatechin (EGC) being more unstable than (−)-epicatechin (EC) and (−)-epicatechin gallate (ECG). Furthermore, they suggested that other ingredients used in production of tea drinks, such as citric acid or ascorbic acid, might interact with green tea catechins and affect their stability.

Recently, based on a mathematical model, Labbé, Tremblay, and Bazinet (2006) highlighted that catechin behaviours during green tea brewing may be divided in two groups, the time-dependent compounds (EGC and EC) and the time/temperature dependent compounds (EGCG, GCG, ECG). According to this particular diffusion behaviour of catechins, EGC- and EGCG-enriched green tea drinks were produced using a two-step extraction procedure (Bazinet, Labbé, & Tremblay, 2007). These catechin-enriched drinks have the advantages to be produced by brewing in hot water and with no addition of ingredients. However, this preliminary study

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was done on one single green tea, and no test on the catechin stability and extraction yield of these enriched drinks was carried-out. Consequently, the purpose of this present study was to produce EGC- and EGCG-enriched green tea drinks based on the two-step extraction procedure reported by Bazinet et al. (2007) and to verify the catechin extraction yields as well as the catechin stability of those enriched green tea drinks during long-term storage. In order to reach this goal, three main objectives were identified. These included: (1) determination of the total content of major catechins of three different green teas, in order to evaluate the catechin extraction yield of the two-step extraction procedure, (2) assessing the generalization of a sequential two-step extraction procedure for the production of EGC- and EGCG-enriched tea drinks and (3) studying the catechin and caffeine degradations during storage of the EGC- and EGCG-enriched drinks in comparison with a control brewing.

2. Materials and methods

2.1. Materials

2.1.1. Green teas

Three commercially available Japanese green tea samples were gratefully donated by Le Palais des Thés (Paris, France). The Ryokucha Midori and Sencha Supérieur leaves were from Shizuoka (Japan) and the Sencha Ariake leaves were from Kyushu (Japan). All teas were harvested in 2005. Green teas were stocked at room temperature in a dark and dry place.

2.1.2. Catechin and caffeine standards

The compounds (−)-epicatechin, (−)-epigallocatechin, (−)-epicatechin gallate, (−)-epigallocatechin gallate, (−)-gallocatechin gallate and caffeine standards were bought from Sigma Company (St.-Louis, MO, USA).

2.2. Methods

2.2.1. Protocols

2.2.1.1. Catechin composition of green tea leaves. The catechin content of the green tea leaves was evaluated using Perva-Uzunalić et al. (2006) solvent extraction method with slight modifications. The water/acetonitrile (50/50, v/v) solution was selected to optimize catechin extraction efficiency (up to 99.8%). Thus, catechins were extracted from four grams of green tea leaves with 200 mL of solvent (a ratio of 1 g for 50 mL (Bazinet et al., 2007) in a round-bottom flask equipped with a condenser and mixed with a magnetic stirrer. The extraction temperature was kept at boiling point for 2 h, then samples were taken and cooled for HPLC analysis. Those extractions were done for each green tea in triplicate.

2.2.1.2. Two-step extraction procedure. The first extraction was done with 20 g of green tea brewed in 1 L of double-distilled water (a 1:50 tea/water ratio was used according to Wang, Helliwell, and You (2000) and our previous experiments (Bazinet et al., 2007; Labbé et al., 2006)) thermostated at 30 °C for 30 min to produce the EGC-enriched tea drink. Thereafter, leaves were gently squeezed to extract water and then transferred for the second brewing step in another litre of double-distilled water which was maintained at 75 °C for 40 min to produce the EGCG-enriched tea drink. A control brewing was also carried-out under the same conditions at 75 °C for 40 min to produce a total catechin-enriched tea drink. Samples of 10 mL were taken at the end of each brewing steps including the control brewing. Each solubilization condition set was repeated thrice for each tea and samples were all cooled quickly and immediately analysed by HPLC.

2.2.1.3. Catechin stability during storage. At the end of the two-step extraction procedure, final brewings (including the control brewing) were used to fill-up five 15 mL tubes. Proper precautions were taken to prevent headspace and thus limit oxygen to that dissolved in the liquid. Also, each tube was opened and analysed only once. Samples were cooled at 4 °C and kept in the dark. HPLC analyses were carried-out after 1, 2, 3, 4 and 8 weeks of storage.

2.2.2. HPLC method

Each green tea sample collected during brewing under different conditions was filtered through a 0.20 μm filter (Aerodisc LC13 PVDF, Gelman Laboratory, Ann Arbor, MI) and diluted by a factor of 10 with HPLC grade water to be analysed. The mobile phases were filtered through a 0.20 μm nylon filter (Mandel Scientific Company, Guelph, ON, Canada). The column temperature was maintained at 25 °C during analyses and autosampler temperature was kept at 4 °C. The detection of analytes was performed with UV detection at 210 nm. Standard curves were calculated from a mix of catechin and caffeine compounds at different concentrations: correlations obtained ranged from 0.99963 to 0.99997. The pump used was a WatersTM 600 pump, the detector was a WatersTM 486 Tunable Absorbance Detector, the autosampler was a Waters 717 plus one and the software was Millennium32 v3.20 (Waters Inc., Lachine, QC, Canada). The column used was an YMC-Pack ODS-AM column, S-5 μm, 12 nm (YMC Inc., Milford, MA, USA) and solvents were water +0.05% trifluoroacetic acid (TFA, purity >99%, Laboratoire MAT, Québec, QC, Canada) for phase A and acetonitrile (HPLC grade, EMD Chemicals Inc., Gibbstown, NJ, USA) + 0.05% TFA for phase B. All other parameters of the HPLC method were the same as those used by Labbé, Araya-Farias, Tremblay, and Bazinet (2005) which were based on the National Institute of Standards and Technology method (Dalluge, Nelson, Thomas, & Sander, 1998).

3. Results and discussion

3.1. Catechin composition of green tea leaves

According to the acetonitrile extraction, Ryokucha Midori tea showed an overall content in major catechins (EGC, EC, EGCG, GCG and ECG) higher than the other teas with a total of 3314.7 μg/mL (Table 1). Sencha Ariake was richer than Sencha Supérieur with major catechin contents of 2713.1 μg/mL versus 2487.8 μg/mL. EGCG and ECG concentrations followed the same trends as observed for the total content in major catechins. EGC concentration was the highest one in Ryokucha Midori, higher than in Sencha Ariake and Sencha Supérieur. EC content in Ryokucha Midori was higher than the two other tea samples which were found to be similar. Finally, GCG concentration was similar in the Ryokucha Midori and Sencha Ariake teas which were higher than the Sencha Supérieur tea.

Even if they were all processed in a similar way (they are all Sencha), their particle sizes were similar and they were extracted

Table 1
Catechin content (μg/mL) of three different green teas obtained by a 50/50 water/acetonitrile extraction at boiling point during 2 h with a 1:50 tea/solvent ratio

	Ryokucha Midori		Sencha Ariake		Sencha Supérieur	
	Mean	±SD	Mean	±SD	Mean	±SD
EGC	969.3	57.3	853.8	16.1	934.6	29.2
EC	197.5	10.3	177.6	9.2	181.7	5.6
EGCG	1633.5	97.9	1224.5	38.4	1042.8	35.6
GCG	156.7	21.2	156.7	11.1	81.5	1.0
ECG	357.7	21.8	300.6	13.4	247.3	14.5

following the same protocol, the different variations between teas are quite realistic. In fact, catechin contents are influenced by non-controllable variables such as the tea vintage, the soil for tea cultivation or the exposition to the sun (Kumamoto & Sonda, 1998). The time of the harvest is also very important as tea leaves harvested in the summer are usually richer in polyphenols (which includes catechins) than those harvested in the spring (Demeule et al., 2002). Peterson et al. (2005) highlighted such variations as they reviewed the catechins concentration obtained from water extraction of green tea leaves and reported EGCG variations ranging from 1182 to 18,810 mg/100 g of dry tea leaves.

3.2. EGC- and EGCG-enriched tea drinks

In order to determine the best parameters to produce EGC- and EGCG-enriched tea drinks, each tea sample was subjected to a catechin and caffeine solubilization kinetics study using a range of time (5, 10, 20, 40 and 80 min) and temperature (30, 45, 60, 75 and 90 °C) combinations. Data of diffusion kinetic for different catechins and also for caffeine were submitted to the 3D model previously developed by Labbé et al. (2006), and it appeared, even if the total catechins and caffeine concentration extracted may differ, that solubilization kinetics of these compounds were very similar and may be well described by the model (R^2 ranging from 0.791 to 0.932, data not shown). According to these results, parameters of the first step extraction, meant to extract the maximum EGC content while keeping most of the EGCG in the leaves, were fixed at 30 °C for 30 min. The second step extraction, aiming the extraction of the maximum EGCG content, was fixed at 75 °C for 40 min in order to avoid an EGCG decrease occurring at 90 °C (data not shown).

According to our HPLC analysis, it appeared that catechin and caffeine contents obtained after the first and second extraction step were different. As expected, EGC represented most of the catechins content of the first extraction step, with values ranging from 55.6% to 63.4% and 66.7% while its concentration decreased to 23.2%, 33.8% and 43.5% in the second extraction step for the Ryokucha Midori, the Sencha Ariake and the Sencha Supérieur, respectively (Table 2). On the opposite, EGCG content was lower in the first extraction step compared to the total catechins extracted, 30.3%, 22.5% and 18.5%, and higher in the second with values of 55.5%, 48.6% and 41.0% still for the Ryokucha Midori, the Sencha Ariake and the Sencha Supérieur, respectively (Table 2). These results are similar to the relative EGC/EGCG concentrations obtained by Bazinet et al. (2007). Although they reported a relative EGC concentration of 78.9% in the EGC-enriched fraction which was higher than our current results, the relative EGCG concentration of 47.6% in the second fraction was comparable to the Sencha Ariake current EGCG yield.

Table 2
Ryokucha Midori, Sencha Ariake and Sencha Supérieur catechin and caffeine concentrations (μg/mL) after the first and second step of the two-step extraction procedure and after a control brewing

	1st step 30 °C – 30 min		2nd step 75 °C – 40 min		Control brewing 75 °C – 40 min	
	Mean	±SD	Mean	±SD	Mean	±SD
Ryokucha Midori						
EGC	676.1	8.2	245.0	8.0	797.2	33.6
Caff	389.5	19.5	257.5	21.2	526.1	21.2
EC	118.0	8.3	42.5	15.0	140.5	7.8
EGCG	368.2	24.4	585.8	31.5	908.9	60.6
GCG	0.0	0.0	50.0	27.8	44.9	3.7
ECG	53.0	3.2	132.8	51.1	160.6	10.8
Sencha Ariake						
EGC	565.1	113.0	317.1	20.9	834.5	60.7
Caff	259.7	25.7	235.7	49.2	491.4	36.9
EC	96.1	15.4	57.9	9.3	152.7	2.6
EGCG	200.2	59.8	455.8	18.1	722.2	28.7
GCG	0.0	0.0	19.5	16.9	37.6	2.9
ECG	29.8	10.0	86.7	13.3	140.2	8.5
Sencha Supérieur						
EGC	569.6	44.5	347.3	10.1	843.6	66.0
Caff	211.6	19.5	179.9	10.1	377.8	21.2
EC	99.8	7.5	63.9	3.8	157.6	6.4
EGCG	157.9	19.5	327.3	8.7	517.6	28.2
GCG	0.0	0.0	0.0	0.0	28.1	1.6
ECG	26.5	4.6	60.4	1.4	102.5	6.2

However, EGCG did not represent the most abundant catechin in the second brewing of the Sencha Supérieur tea as for the others. This might be easily explained by the fact that the Sencha Ariake possesses approximately the same content in EGC as the two other teas with a lesser amount in EGCG. Consequently, the unextracted EGC in the first brewing still represents a large part of the catechin content of the second brewing since less EGCG can be extracted (Table 1). Overall, EGC absolute concentration decreased by 63.8% (676.1–245.0 μg/mL), 43.9% (565.1–317.1 μg/mL) and 39.0% (569.6–347.3 μg/mL) in the second brewing while EGCG absolute concentration was 1.59 (368.2–585.8 μg/mL), 2.28 (200.2–455.8 μg/mL) and 2.07 (157.9–327.3 μg/mL) fold higher for Ryokucha Midori, Sencha Ariake and Sencha Supérieur teas, respectively (Table 2). The results for the three other catechins analysed were quite similar for the three teas as EC was mostly extracted in the first brewing and GCG and ECG were found to be more abundant in the second, except for the Sencha Supérieur which GCG content was below the detectable limit. As a good part of caffeine content was successfully extracted with the first step extraction, caffeine values were lower or slightly lower in the EGCG-enriched fraction (Table 2).

These results confirm that it is possible to produce EGC- and EGCG-enriched tea drinks regardless of the green tea used based on the previously developed two-step extraction method (Bazinet et al., 2007). In fact, good extraction efficiencies were obtained for the first extraction step with extraction yields ranging from 67.5% to 84.8% of the water extractable EGC and for the second extraction step with an average extraction yield of 63.6% for the water extractable EGCG compared to the control brewing at 75 °C for 40 min (Table 2). In comparison with the water/acetonitrile extraction, the average EGC extraction yield for the three teas in the first extraction step was 65.6 ± 3.6% while the average EGCG extraction yield during the second extraction step was 34.8 ± 2.5%. The EGCG extraction yield was relatively low in comparison with the water/acetonitrile, but in the case of a nutraceutical drink, the use of water is preferred to an organic solvent.

3.3. Catechin and caffeine long-term stability

HPLC analyses were performed to assess whether or not the catechin and caffeine contents of the EGC- and EGCG-enriched tea drinks compared to a control brewing were subjected to degradation when stored for few weeks without addition of any preservative or antioxidant agent.

According to our results, no catechin or caffeine significant degradations occured in the two enriched drinks or in the control brewing. As the catechin and caffeine concentration was not subject to variation during the 8 weeks of storage, the HPLC results obtained during the experiment (at week 0, 1, 2, 3, 4 and 8) were pooled together as shown in Table 3.

Several studies reported that many factors may affect catechins or more precisely EGCG stability including pH, temperature, oxygen level, antioxidant level, metal ions and the concentration of other ingredients in tea, including catechins (Chen et al., 2001; Sang, Lee, Hou, Ho, & Yang, 2005; Su et al., 2003; Wang, Zhou, & Wen, 2006). Catechins were founds to be more stable at acidic

Table 3
Mean catechin and caffeine concentrations (μg/mL) of Ryokucha Midori, Sencha Ariake and Sencha Supérieur over 8 weeks of storage at 4 °C

	1st step 30 °C – 30 min			2nd step 75 °C – 40 min			Control brewing 75 °C – 40 min		
	μg/mL	SD	%CV	μg/mL	SD	%CV	μg/mL	SD	%CV
Mean data on 8 weeks (n = 18)[a]									
Ryokucha Midori									
EGC	**685.7**	9.8	1.4	**258.7**	8.2	3.2	**814.2**	13.7	1.7
Caff	387.2	6.2	1.6	258.1	9.2	3.6	553.2	18.2	3.3
EC	125.9	6.7	5.3	48.2	3.9	8.1	151.1	9.6	6.4
EGCG	**352.2**	25.0	7.1	**561.8**	26.9	4.8	**900.1**	36.3	4.0
GCG	0.0	0.0	0.0	44.7	7.4	16.4	48.0	2.2	4.6
ECG	51.2	5.1	10.0	110.6	16.1	14.5	160.0	10.9	6.8
Sencha Ariake									
EGC	**580.3**	9.1	1.6	**329.9**	12.1	3.7	**840.5**	18.1	2.2
Caff	281.8	14.1	5.0	251.0	16.2	6.5	495.4	10.8	2.2
EC	104.9	5.2	5.0	65.1	7.7	11.8	156.9	8.2	5.2
EGCG	**208.3**	12.3	5.9	**469.6**	23.0	4.9	**718.4**	15.9	2.2
GCG	0.0	0.0	0.0	36.8	19.0	51.6	41.5	4.2	10.2
ECG	31.3	3.0	9.4	98.2	20.0	20.4	140.0	8.0	5.7
Sencha Supérieur									
EGC	**578.7**	13.9	2.4	**359.7**	11.0	3.1	**846.7**	16.6	2.0
Caff	221.2	7.6	3.5	192.4	12.8	6.7	382.4	10.0	2.6
EC	105.4	7.2	6.8	66.2	5.3	7.9	161.0	7.0	4.4
EGCG	**146.6**	24.0	16.3	**332.6**	14.9	4.5	**510.6**	23.9	4.7
GCG	0.0	0.0	0.0	3.8	9.2	244.9	31.5	4.1	13.0
ECG	19.2	9.2	48.1	65.1	7.5	11.5	102.3	10.2	10.0

Samples were done in triplicate and analysed after their brewing and after 1, 2, 3, 4 and 8 weeks (n = 18).
[a] Sencha Ariake 8th week data for the 1st step were not available, thus n = 15.

pH rather than alkaline (Su et al., 2003) and lower storage temperature extended appreciably their half-life (Demeule et al., 2002). Higher oxygen levels and low concentration of antioxidants increased catechin oxidation while the presence of metal ions enables a metal-catalyzed auto-oxidation of EGCG (Sang et al., 2005). Finally, the presence and concentration of other ingredients such as sucrose, citric acid and ascorbic acid enhanced the degradation of catechins in a solubilized purify green tea catechins extract (Su et al., 2003). However, a higher catechin concentration was found to positively extend the shelf life of EGCG and other catechins (Sang et al., 2005).

As no significant degradation of the catechins occurred during the 8 weeks of storage, the balance of the factors affecting stability must have been positive. As a matter of fact, the use of a double-distilled water prevented the presence of extrinsic metal ions in the brewing and the proper precaution taken to prevent headspace in the 15 mL tubes limited oxygen only to the dissolved molecules. An acidic pH of around 5.5, a storage temperature of 4 °C also contributes in the limitation of the catechins degradations. Finally, as the EGC- and EGCG-enriched fractions are not pure catechins extracts, the presence of other catechins probably highly contributes to the stability of the catechin content.

Those results are quite promising considering that green tea catechin from the EGC- and EGCG-enriched tea drinks, were not expected to be stable over more than few weeks. In fact, Su et al. (2003) reported degradation as high as 78% of the green tea catechins maintained in a sodium buffer at a pH of 5 over only 1 month. Further experiments are currently under way to determine, in commercial conditions of production, the chemical and microbial long-term stability of those enriched tea drinks and the different factors which may positively or negatively influenced them.

4. Conclusions

In this study, it was demonstrated that the previously developed two-step extraction procedure to produce EGC- and EGCG-enriched green tea drinks (Bazinet et al., 2007) may be generalized to other green teas. Considering the catechins great stability in the EGC- and EGCG-enriched tea drinks, on an industrial point of view, this simple two-step extraction procedure could be considered as an interesting way to produce enriched green tea drinks with more potent and stable bioactive catechins than pure catechins extracts which are often costly and time consuming.

The two-step extraction procedure for the production of catechin-enriched tea drinks has many advantages in comparison with extraction procedures currently used. The extraction of the two main catechins (EGC and EGCG) can be controlled since EGCG extraction is more time/temperature dependent than EGC. The procedure is an environmental method since no organic solvent is used, water being the extraction medium, and the extraction yield in comparison with water extraction are realistic. Finally, this two-step extraction procedure can be easily scaled-up and used at industrial scale.

A clinical study is currently under way to test the EGCG-enriched tea drink, produced in the second step of the two-step extraction procedure, on the maintenance of complete remission in woman with advanced ovarian cancer.

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