International Journal of Secondary Metabolite: Vol. 3, Issue 1, 2016, pp. 3-13

Research Article

ISSN:2148-6905 online Journal homepage: http://www.ijate.net/index.php/ijsm

New approaches for effective microwave assisted extraction of caffeine and catechins from green tea

Gönül SERDAR[1], Ezgi DEMİR[1], Serhat BAYRAK[1], Münevver SÖKMEN[1,*]

[1]Karadeniz Technical University, Faculty of Science, Department of Chemistry, 61080 Trabzon, Turkey

Received: 3 March 2015 - Revised: 4 May 2015- Accepted: 20 June 2015

Abstract: This study was designed to develop an effective microwave assisted extraction (MAE) method for extraction of caffeine and catechins from green. A series of solvents (*water, ethanol:water mixture or citric acid:water mixture*) were used for extraction of green tea samples (*fresh, frozen or dried*) collected in three collection periods (*first, second and third collection periods*). Samples were extracted in a close microwave system under the certain conditions. Extraction was carried out under a controlled 600 W microwave power at 80 ^{o}C temperature for 4 min irradiation time. Alternative to water, an ethanol-water mixture (1:1) or a citric acid-water (1:1) mixture was used as extracting solvent under the same conditions. After MAE crude aqueous extract was partitioned first with chloroform to separate caffeine then ethyl acetate for catechins. Both caffeine and catechin extraction was quite successful with microwave assisted system employing only 4 minutes treatment. Ethanol-water mixture seems to be appropriate for effective extraction in the basis of extract yields. However, HPLC results showed that individual catechin content of each extraction is more important criteria for the evaluation of most effective extraction medium rather than the mass of the extract. Using citric acid as extracting solvent in MAE seem to be more fruitful providing 100 % catechin mixture with the highest EGCG content.

Key words: Caffeine extraction, catechin extraction, microwave assisted extraction

1. Introduction

Tea is the most consumed drink in the world and produced in large scale in many countries. It is consumed mainly as black tea but green tea and other tea types such as poorer or oolong consumption is increasing. Green tea refers to non-fermented tea and contains tea polyphenol, caffeine, amino acids, saponins, tannins, etc., with about 10-30 % (w/w) polyphenols and 2-4 % (w/w) caffeine (Pan et al., 2003). Tea polyphenols involves catechines, flavanols, phenolic acids, flavanonens, glycosides and plant pigments (Wang et al., 2010). The catechins can be categorized into two groups based on their structure: epistructured catechins and non-epistructured catechins. The epistructured catechins are consisted of epigallocatechin (EGC), epicatechin (EC), epigallocatechin gallate (EGCG), and epicatechin gallate (ECG), of which EGCG is the major constituent and the most powerful one; as the non-epistructured catechins are gallocatechin (GC), catechin (C), gallocatechin gallate (GCG), and catechin gallate (CG) (Vuong et al., 2010).

Conventional solid-liquid extraction methods are used for effective extraction of caffeine and catechines. These methods are easy but require long extraction periods and energy. Row and Jin (2006) proposed a liquid extraction method for Korean green tea samples. Green

[*]Corresponding Author Phone:+90-462-377-25-32; Fax: :+90-462-325-31-96 E-mail: msokmen@ktu.edu.tr

tea leaves had been extracted in water at varying temperatures (50, 80, 100 ^{o}C) and extraction periods (4 hours, 40 minutes and 15 minutes). After filtration supernatant was extracted by chloroform (1:1 v/v) to separate caffeine then following this aqueous phase was re-extracted with ethylacetate (1:1 v/v) to separate catechins. They report that the highest yield was obtained with extraction at 80 ^{o}C for 40 minutes.. In another study, Wang et al. (2009) applied a boiling water extraction first then after cooling the water phase was extracted with ethyl acetate to separate catechins. Vovk et al. (2005) have followed a similar extraction but using chloroform to remove chlorophyl first then ethyl acetate for extraction of catechins. Apart from these studies Goodarznia and Govar (2009) have employed a superheated water extraction of green tea leaves. In 2011 Vuong et al. published an important review related to extraction techniques of tea samples. All extraction techniques used for effective extraction of tea samples have been discussed in details.

Microwave assisted extraction (MAE) is a new technique that provides lower extraction period and less energy consumption. Apart from these microwave produces high temperature regions that ease the disruption of the cell wall. As a result of this effect, active compounds are more quickly extracted into the solvent. Water can be used for controlled temperature MAE of tea principles. Pan et al. (2003) have developed a simple procedure for effective extraction of catechins and caffeine from green tea leaves. A portion of dry tea was pre-leached with 50 % v/v ethanol at room temperature for 90 min at a ratio of solvent to tea of 20:1 (mL/g). Microwave extraction procedure was applied over 4 minutes as following: initially on for 45 s, then off for 10 s and then continuously on for 3 s and off for 10 s over the next 3 min, with the aim of maintaining the extraction temperature around 80 ^{o}C. Recently Nkhili et al. (2009) has reported a study for extraction tea using a 600 W microwave oven. The temperature was controlled between 80 and 100 ^{o}C for 30 min and the solvent to tea ratio was 20:1 (mL/g). Under these conditions the extraction efficiency for the tea catechins was higher than for the conventional heating methods.

This study was designed to develop an effective microwave assisted extraction (MAE) method for extraction of caffeine and catechins from green tea. A series of solvents (*water, ethanol:water mixture or citric acid:water mixture*) were used for extraction of green tea samples (*fresh, frozen or dried*) collected in three collection periods (*first, second and third collection periods*). Tea samples were extracted using water in a close microwave system under the certain extraction conditions. Alternatively two more extraction procedures were applied in this study. An ethanol-water mixture (1:1) or citric acid-water (1:1) mixture was used as extraction solvent under the same conditions. Presence of an organic solvent or citric acid expected to increase the extraction ratio of phenolic compounds. Tea is collected in large quantities therefore the nature of the samples is another important parameter for industrial applications. Fresh, frozen or dried tea samples might have variations in quantitative yield or constituents of the extracts. This is the first report employing MAE method for extraction, isolation and quantification of green tea that is grown in eastern Black Sea region collected in different collection periods.

2. Materials and methods

2.1. Chemicals

Methanol, ethanol, ethyl acetate and chloroform were analytical grade from Merck. The standard chemicals; caffeine, catechin (C), (−)epicatechin (EC), (−)epigallocatechin (EGC), (−)epigallocatechin gallate (EGCG), were purchased from Sigma Aldrich.

2.2. Green tea samples

Green tea used in the experiments was supplied by Sürçay San. Co. Ltd. (Sürmene, Trabzon). In Turkey, tea is collected three times in a year. First collection is in May, second is in June, and the third is in August-September. Green tea samples used in our tests were collected from the same tea garden at each collection period in 2013. Leaves on top branches of tea plant was collected and placed in a sealed plastic bag immediately transferred to laboratory. Tea undergo quick enzymatic reactions therefore should be processed immediately. When large amount of tea processing is considered drying or freezing might be alternative storage. Therefore three sample preparation methods were designed. The first group green tea leaves was left drying at room temperature, second group was fresh green tea sample chopped in a blender just after collection, and the last group was stored at -20 ^{o}C for a certain period then chopped as above.

2.3. Microwave extraction of the samples

Tea catechins and other components were extracted using a close microwave assisted extraction system (Milestone, Start S Microwave, USA). MAE parameters such as microwave power and extraction time can affect the extraction efficiency (Deng et al., 2006). Therefore, optimized extraction procedures given in the literature were modified for an effective extraction. Citric acid-water mixture was first time applied in MAE of tea samples.

(i) W*ater extraction*: 10 g of tea sample (dry, fresh or frozen) and 200 mL distilled water were placed in vessel and shaken for 90 minutes at room temperature. Then sample was transferred into microwave extraction apparatus (Pan et al., 2003). Extraction was carried out under a controlled 600 W microwave power at 80 ^{o}C temperature for 4 min irradiation time (Nkhili et al., 2009). After extraction, the flask was allowed to cooling down to room temperature before opening the cap.

(ii) Ethanol extraction: 10 g of tea sample (dry, fresh or frozen) and 200 mL ethanol-water (1:1 v/v) solution were placed in vessel and shaken for 90 minutes at room temperature. After pretreatment step the sample was extracted as described above.

(iii) Citric acid extraction: 10 g of tea sample (dry, fresh or frozen) and 200 mL (1:1 v/v) citric acid (0.1 M)-water solution were placed in vessel and shaken for 90 minutes at room temperature. After pretreatment step sample was extracted as described above.

All extracts were filtered through 110 mm filter paper and filtrates were initially partitioned with chloroform to remove caffeine. The water phase was collected and the impurities associated with the chloroform phase were discarded. Then aqueous phase was extracted with ethyl acetate three times using 150 mL ethyl acetate in each extraction to separate catechins. The chloroform and ethyl acetate phases were filtered and the filtrate was concentrated by a rotary evaporator under reduced pressure at 55 ± 2 ^{o}C.

2.4. Quantification of caffeine and catechins

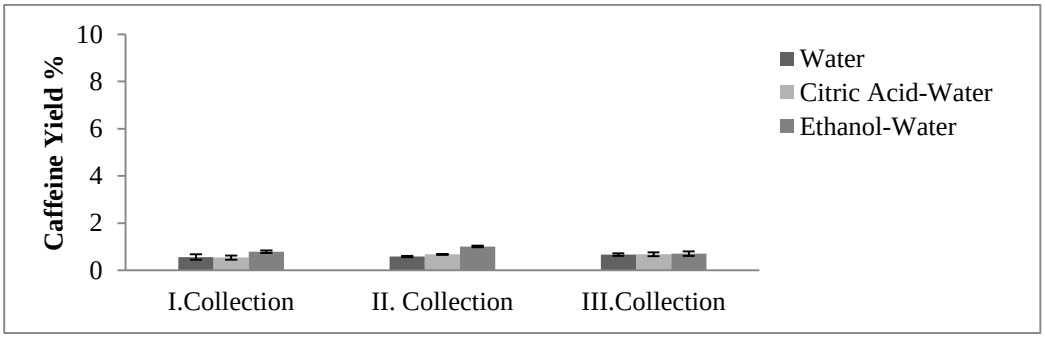
Quantification of caffeine and catechins were done by weighting the chloroform and ethyl acetate fractions after evaporation the solvent and drying the extract. Additionally HPLC analyses were carried out to determine the amount of each catechins in the mixture. The instruments used in this study was a HPLC system (Hitachi Elite Lachrom, Japan) equipped with a Shim-pack VP-ODS C18 column (5 mm, 4.6 x 250 mm, 35 ^{o}C) at 278 nm. Solvents A (water) and B (DMF-methanol-acetic acid mixture, 20:1:0.5) were run with 86 % A for 13 minutes then its volume was decreased to 64 % within next 15 minutes and finally back to initial concentration for another 6 min. Concentrations of catechins were quantified by their peak areas against those of standards prepared from original compounds (Wang et al., 2011).

3. Results and discussion

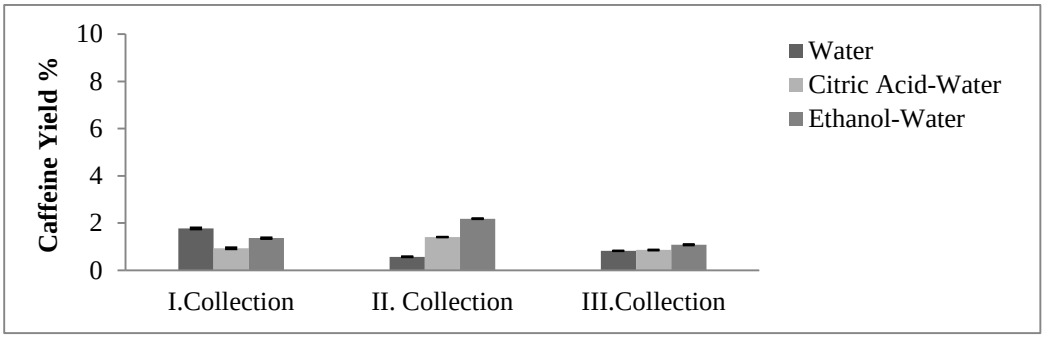
3.1. Extraction yields

Results given in this study were obtained from the samples collected in 2013. The average yields of caffeine from the first (in May, *I. Collection*), second (in June, *II. Collection*) and third collection periods (in August-September, *III. Collection*) are given in the Figure 1.

a) Fresh



b) Frozen



c) Dry

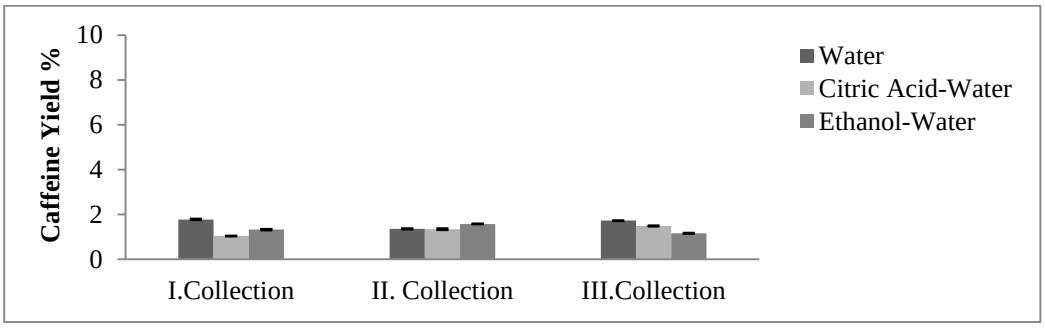
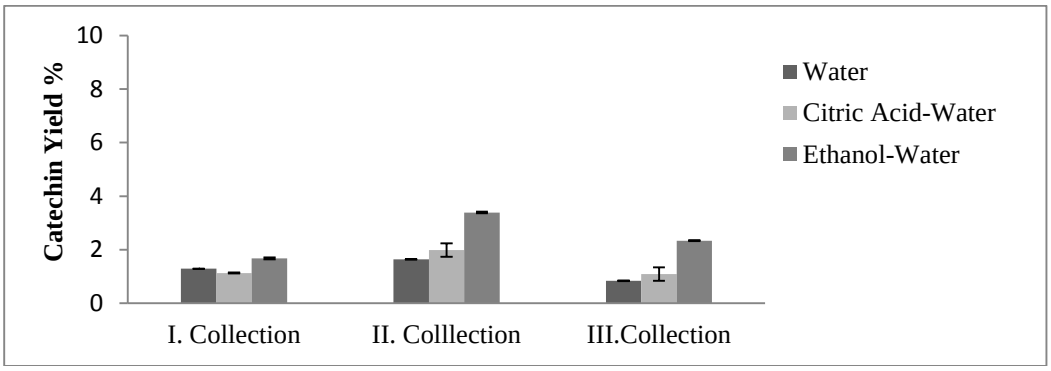


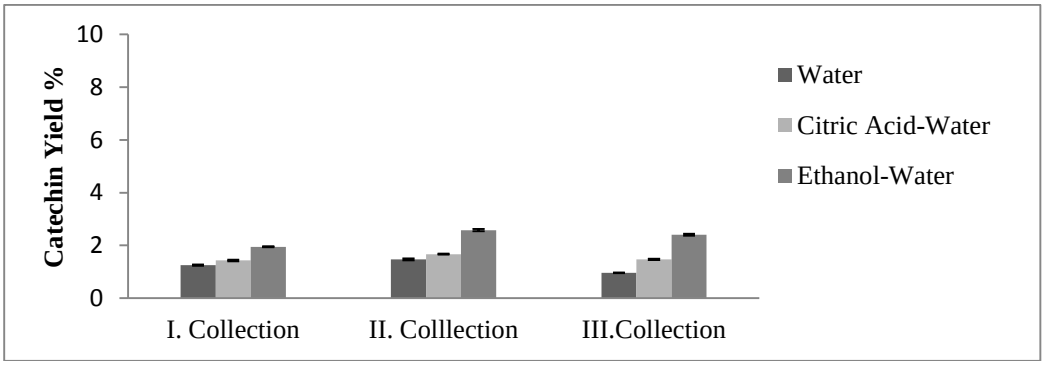
Fig. 1. Comparison of caffeine content of the fresh, frozen and dry green tea leaves in three collection periods using different solvents.

In general, dried tea samples provide higher caffeine yields. This is expected since drying naturally decrease the water content and increase the concentration of the chemicals. Fig. 1 shows that ethanol-water solution give higher caffeine extract than water or citric acid solution. Mass of caffeine extract was slightly high in second collection period. Catechin yields calculated from ethyl acetate extractions are given in Figure 2.

a) Fresh



b) Frozen



c) Dry

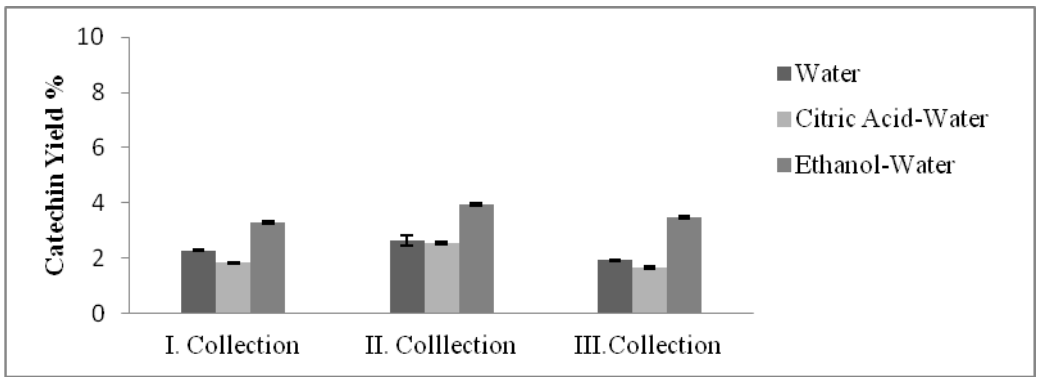


Fig. 2. Comparison of total catechin content of the fresh, frozen and dry green tea leaves in three collection periods using different solvents for extraction.

The mass of catechin extracts was always higher than caffeine. Microwave energy penetrates materials and produces a heat zones in materials to be extracted. During this process polar solvents and dissolved ions produce a molecular friction resulting from dipolar rotation and the conductive migration (Oliveira and Franca, 2002). The temperature is not distributed equally and localized in zones so ease the selective migration of target compounds from the material in shorter time. Using water as extracting solvent provides lower extract yields and presence of citric acid seems to be not enhancing the extraction yield. The highest catechin content was obtained from dried samples that were extracted by ethanol-water (1:1 v/v) solution. Similar to caffeine results second collection period provides the highest catechin yield. Interestingly, drying process results 65-70 percent loss of water content of green tea and total catechin content should be 2-3 times higher than fresh or frozen leaves. However our results are not in accordance with this expectation. Drying results more stiff plant material that chemicals in the material not be easily extracted into the solution in a short time such as 4 min. It is clear that water containing fresh material is easing the microwave extraction. This should

be related to water content of the biomaterial and temperature zones created by microwave. As a result of this effect the possibly cell wall was easily destroyed to release the catechins into solvent.

Weighting extract mass can give a rough idea about how much catechins can be isolated with solvent used for the extraction. But partition of catechins from microwave assisted extract is not purely selective and ethyl acetate can remove some other principles as well as catechins. Therefore, the best strategy to evaluate the most suitable solvent for extraction is to carry out a quantification study of individual catechin present in the extract.

3.2. HPLC Analysis of the green tea extracts

Caffeine and catechin constituents of the extracts were determined by reversed-phase HPLC. Catechin standards were catechine (C), epicatechin(EC), epigallocatechin (EGC) and epigallocatechin gallate (EGCG). The chromatogram of the standard mixture is given in Figure 3.

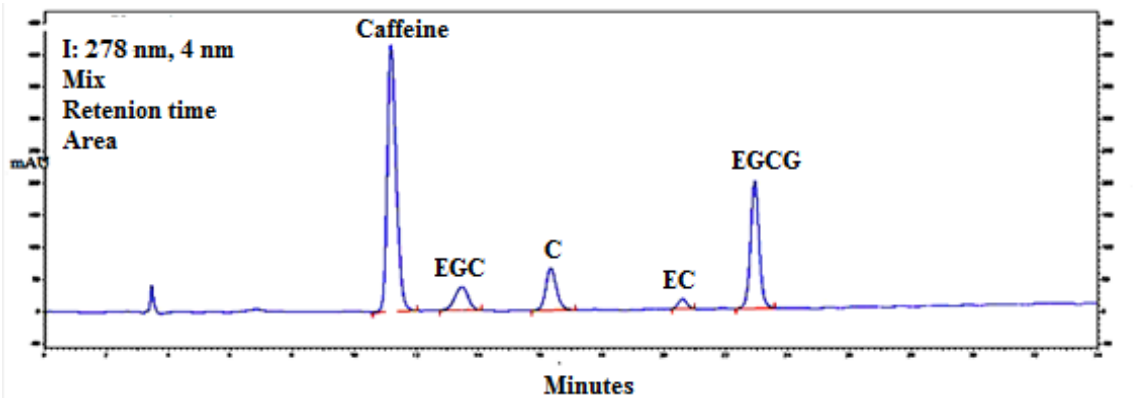


Fig. 3. The chromatogram of caffeine and catechin standarts. Retention times are as below; caffeine 11,18; EGC 13,34; C 16,44; EC 20,60; EGCG 22,03 min. Injection volume is 20 µL

As seen from Figure 2, the extract yields of fresh, frozen and dried samples exhibited variations as well as collection periods. The highest catechin constituent for first, second and third collection periods had been obtained with ethanol-water mixture. HPLC chromatograms of catechin extracts obtained from fresh green tea samples that was extracted with ethanol-water solvent (for all collection periods) are given in Figure 4. The identification of catechins was carried out by comparing their retention times to standards and the amount of individual catechin was calculated from these chromatograms.

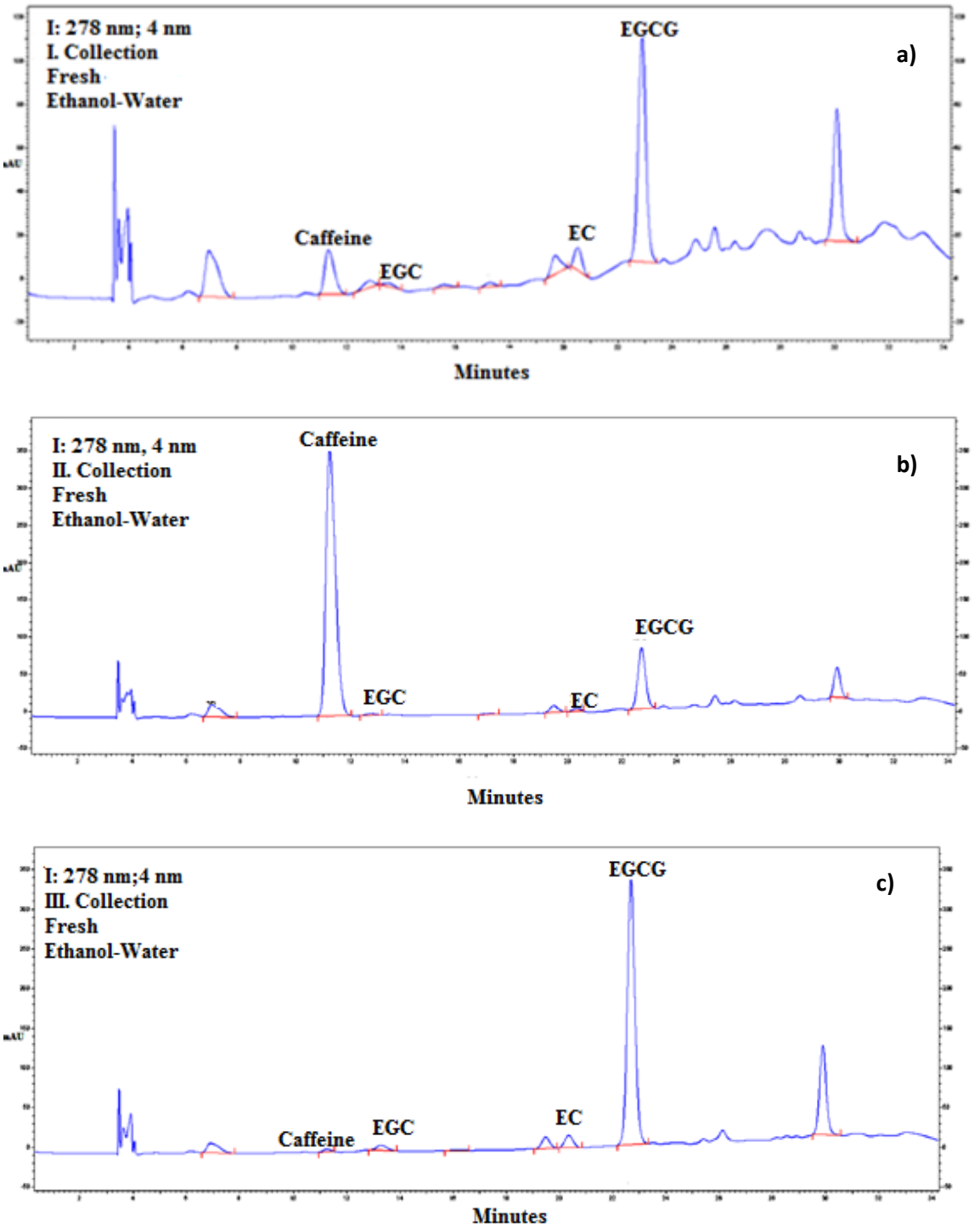
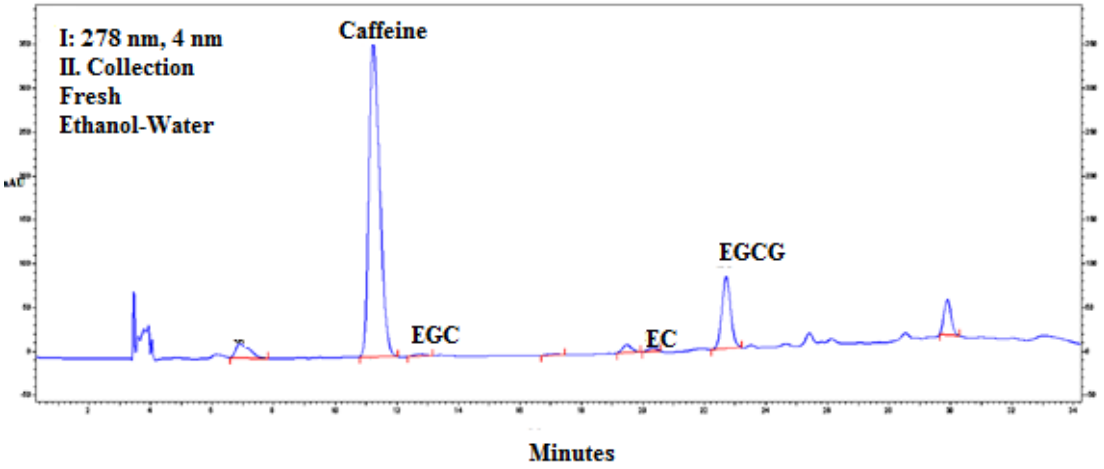


Fig. 4. HPLC chromatogram of catechin extracts obtained from fresh samples using ethanol-water mixture at **a)** first, **b)** second and **c)** third collection periods (600 W microwave power at 80 °C temperature for 4 min irradiation time).

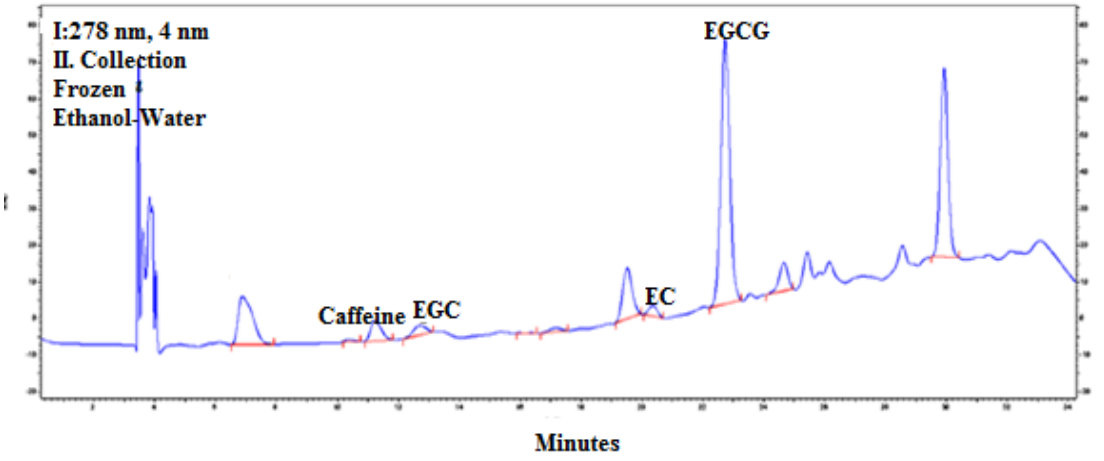
Although the mass of catechin extract was highest in second collection period (3.39 %) its catechin content was not that much high. It is clear that individual catechin content especially EGCG content is higher in the first and third collection periods. It should be noted that individual or total catechin content is more important than total extract mass.

For a good evaluation of sample nature, catechin content of fresh, frozen and dried tea samples (second collection period, ethanol-water extraction) were compared. Related chromatograms are given in Figure 5.

a) Fresh



b) Frozen



c) Dry

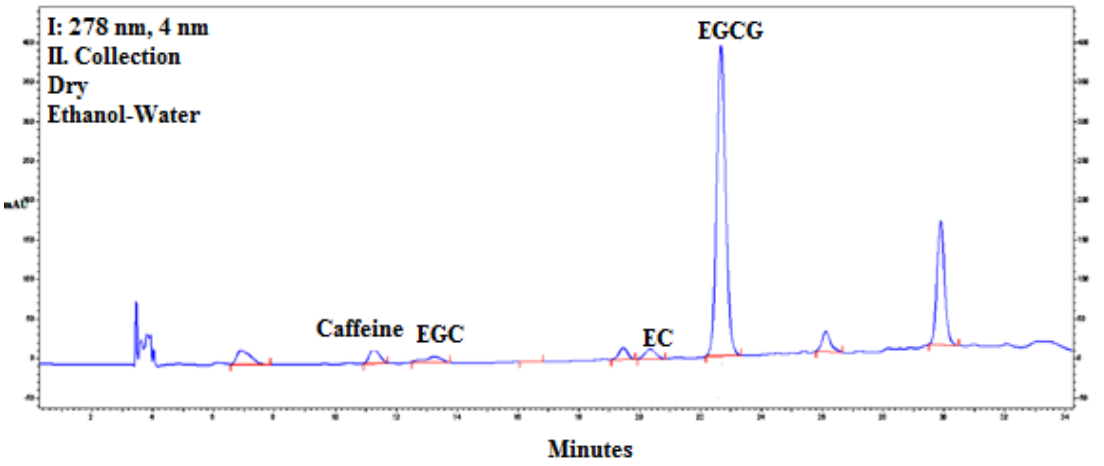


Fig. 5. HPLC chromatograms of catechin extracts obtained from fresh, frozen and dry samples collected in second collection period (using ethanol-water mixture, 600 W microwave power at 80 ºC temperature for 4 min irradiation time).

Both frozen and dried tea samples provide higher EGCG content than fresh samples. EGCG was 10.75 % and 9.63 % of catechin extract for fresh and frozen samples, respectively. However, EGCG was 52.79 % of total catechin extract for dried samples. The quantitative amount of each catechins (as percentage of each extract) are given in Table 1.

Table 1. Quantification of catechins by HPLC analyzes

	I. Collection								
	Dry			Fresh			Frozen		
Catechin (%)	W	CA-W	E-W	W	CA-W	E-W	W	CA-W	E-W
EGC	3.890	1.930	7.300	17.800	3.570	2.150	2.020	4.450	7.170
C							5.370		
EGCG	13.32	7.20	14.63	39.89	40.20	16.77	15.9	9.22	27.98
EC	17.10	4.94	33.56	1.19	18.01	13.59	0	11.23	37.59
Total %	34.31	14.07	55.49	58.88	61.78	32.51	23.29	24.90	72.74
	II. Collection								
	Dry			Fresh			Frozen		
Catechin (%)	W	CA-W	E-W	W	CA-W	E-W	W	CA-W	E-W
EGC	16.240	0.560	6.170	0.720	4.470	0.720	1.210	1.600	1.920
C									
EGCG	4.80	6.43	52.79	4.91	17.37	10.75	6.29	1.98	9.63
EC	73.08	35.20	25.44	0.55	14.04	6.30	3.68	4.30	4.60
Total %	94.12	42.19	84.4	6.35	35.88	17.77	11.18	7.88	15.95
	III. Collection								
	Dry			Fresh			Frozen		
Catechin (%)	W	CA-W	E-W	W	CA-W	E-W	W	CA-W	E-W
EGC	5.500	2.660	2.010	7.160	7.350	4.330	0.100	0.190	0.320
C									
EGCG	24.67	7.49	41.66	41.50	52.45	43.97	1.55	0.69	0.69
EC	2.64	0.34	6.78	45.08	42.82	28.13	1.49	0.45	0.57
Total %	32.81	10.46	50.45	93.74	100	76.43	3.14	1.33	1.58

W: Water
CA-W: Citric Acid-Water
E-W: Ethanol-Water

Table 1 exhibited quite interesting results. Actually, extract yields given in Figure 2 seem to be misleading us. Although extract mass is high its real total catechin constituent might be lower. For example, extraction of fresh leaves (collected in second collection period) with ethanol-water mixture yields 3.39 % extract but only 17.77 percent of this mass is actually catechins. On the other hand, citric acid extraction of the same sample gave only 1.99 % extract yield but 35.88 percent of this mass is catechins. It is clear that using fresh tea samples for extraction is more convenient and highest catechin yields were obtained in third collection period using citric acid solution.

As seen from HPLC chromatograms trace amount caffeine are still present in the extracts and it was also quantified and given in Table 2. Caffeine residue in catechin extracts varies between 0.16-1.68 % of total catechin extract. It means that caffeine is successively removed with chloroform and only small fraction remained in the extract. However, HPLC analyses revealed that caffeine extract is almost pure and has no other compounds.

Table 2. Quantitative analysis of caffeine present in catechin extracts

	Dry			Fresh			Frozen		
Caffeine (%)	W	CA-W	E-W	W	CA-W	E-W	W	CA-W	E-W
I. Collection	1.18	0.39	0.45	0.84	0.90	1.29	1.19	0.42	0.18
II. Collection	0.90	0.94	1.06	0.21	0.24	1.13	0.45	0.80	0.34
III. Collection	1.68	0.64	0.21	0.40	0.67	0.16	0.28	0.34	1.16

W: Water
CA-W: Citric Acid-Water
E-W: Ethanol-Water

This study provides useful information related to effective MAE of green tea samples. Three extraction solvents yielded 0.54-2.15 % of caffeine. The highest caffeine yield was obtained in the second collection period using frozen samples and ethanol-water as extracting solvent. Both caffeine and catechin extraction was quite successful with microwave assisted system employing only 4 minutes treatment. Catechin extract yields were between 0.84-3.96 % depending on the solvent system used.

4. Conclusion

In general, ethanol-water mixture seems to be appropriate for effective extraction in the basis of extract yields. However, individual catechin content of each extraction is more important criteria for the evaluation of most effective extraction medium rather than the mass of the extract. Using citric acid as extracting solvent in MAE seem to be more fruitful providing 100 % catechin containing extract with the highest EGCG content. MAE is fast, economical and green extraction method for effective separation of caffeine and catechin from green tea. As a conclusion extracting green tea with microwave system provide high caffeine and catechins in only 4 minutes treatment method. Presence of citric acid facilitates the extraction of these valuable chemicals.

Acknowledgements

The authors gratefully appreciate the financial support from Turkish Ministry of Industry (SAN-TEZ Project, Grant No 00932.STZ.2011-1). Thanks also go to tea producers OrÇay, SurÇay, Filiz Çay for their financial support.

5. References

Deng CH, Ji J, Yu YJ, Duan GL, Zhang XM (2006). Fast determination of curcumol, curdione and germacrone in three species of curcuma rhizomes by microwave assisted extraction followed by headspace solid-phase microextraction and gas chromatography–mass spectrometry. J Chrom A 1117:115-120.

Goodarznia I, Govar AA (2009). Superheated water extraction of catechins from green tea leaves: Modeling and simulation. Transactions C: Chem. Chem Eng 16:99-107.

Nkhili E, Tomao V, El Hajji H, El Boustani E, Chemat F, Dangles O (2009). Microwave-assisted water extraction of green tea polyphenols. Phytochem Anal 20:408-415.

Oliveira MEC, Franca AS (2002). Microwave heating of foodstuffs. J Food Eng 53:347-359.

Pan X, Niu G, Liu H (2003). Microwave-assisted extraction of tea polyphenols and tea caffeine from green tea leaves. Chem Eng and Process 42:129-133.

Row KH, Jin Y (2006). Recovery of catechin compounds from Korean Tea by solvent extraction. Biores Technol 97:790-793.

Spigno G, De Faveri DM (2009). Microwave-assisted extraction of tea phenols: A phenomenological study. J Food Eng 93:210-217.

Vovk I, Simonovska B, Vuorela H (2005). Seperation of eight selected flavan-3-ols on cellulose thin-layer chromatographic plates. J Chromatogr A 1077:188-194.

Vuong QV, Golding JB, Nguyen M, Roach PD (2010). Extraction and isolation of catechins from tea. J Sep Sci 33:3415-3428.

Wang K, Liu Z, Huang J, Fu D, Liu F, Gong Y, Wu X (2009). TLC seperation of catechins and theaflavins on polyamide plates. J Planar Chrom 22:97-100.

Wang L, Qin P, Hu Y (2010). Study on the microwave-assisted extraction of polyphenols from tea. Front Chem Eng China 4:307-313.

Wang K, Liu F, Liu Z, Huang J, Xu Z, Li Y, Chen J, Gong Y, Yang X (2011). Comparison of catechins and volatile compounds among different types of tea using high performance liquid chromatograph and gas chromatograph mass spectrometer. Int J Food Sci & Techol 46:1406-1412.