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Research Paper / Araştırma Makalesi

Phenolic Content of Some Dietetic Tea Products in Turkey

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ABSTRACT

High performance liquid chromatography (HPLC) system was used to determine phenolic contents of dietetic tea samples (n=10) marketed in Turkey. The most abundant phenolic compounds were gallic acid (ND–6.98 mg/100 g dw), protocatechuic acid (0.98–45.61 mg/100 g dw), 4-hydroxybenzoic acid (ND–31.56 mg/100 g dw), chlorogenic acid (ND–0.73 mg/100 g dw), caffeic acid (11.27–154.55 mg/100 g dw), *p*-coumaric acid (ND–6.56 mg/100 g dw), ferulic acid (ND–1.13 mg/100 g dw), ellagic acid (0.10–1.76 mg/100 g dw), epicatechin (7.59–290.07 mg/100 g dw), rutin (ND–49.95 mg/100 g dw) and quercetin (1.15–10.50 mg/100 g dw). Based on the results of this study, sensitivity and accuracy were good. Calibration curves had a good linearity for all compounds (r^2>0.999). The quantitation limit ranged 0.010 to 0.050 µg/g in dietetic tea products. Recoveries were in the range of 86.45-97.35%. Results had good accuracy and reproducibility.

Keywords: Phenolic compound, Dietetic tea, HPLC

Türkiye'deki Bazı Diyet Çay Örneklerinin Fenolik Bileşik İçeriği

ÖZ

Türkiye'de satılan on diyet çay örneğinin (n=10) fenolik bileşik içeriği, yüksek performanslı sıvı kromatografisi (HPLC) sistemi ile belirlenmiştir. En çok bulunan fenolik bileşikler, gallik asit (ND-6.98 mg/100 g kuru numune), protokateşik asit (0.98-45.61 mg/100 g kuru numune), 4-hidroksibenzoik asit (ND-31.56 mg/100 g kuru numune), klorojenik asit (ND-0.73 mg/100 g kuru numune), kafeik asit (11.27-154.55 mg/100 g kuru numune), *p*-kumarik asit (ND-6.56 mg/100 g kuru numune), ferulik asit (ND–1.13 mg/100 g dw), ellajik asit (0.10-1.76 mg/100 g kuru numune), epikateşin (7.59-290.07 mg/100 g kuru numune), rutin (ND-49.95 mg/100 g kuru numune) ve kuersetindir (1.00 mg/100 g kuru numune). Elde edilen sonuçlara göre, hassasiyet ve doğruluk iyi bulunmuştur. Kalibrasyon eğrileri, tüm bileşikler için iyi bir doğrusallık göstermiştir (r^2> 0.999). Diyet çay ürünlerinde tespit limiti, 0.010-0.050 µg/g arasında değişmiştir. Geri kazanımlar %86.45-97.35 aralığındadır. Bu çalışmada elde edilen sonuçların doğruluk ve tekrarlanabilirliklerinin iyi olduğu belirlenmiştir.

Anahtar Kelimeler: Fenolik bileşik, Diyet çay, HPLC

INTRODUCTION

Phenolic compounds have become valuable compounds of research interest because of their perceived beneficial effects for health, including anti-carcinogenic, anti-atherogenic, anti-ulcer, anti-thrombotic, anti-inflammatory, immune modulating, anti-microbial, vasodilatory, cardioprotective, anti-thrombotic, and analgesic effects [1-6].

The U.S. Food and Drug Administration (FDA) arranges dietary supplement products and dietary ingredients. In

1994, the US congress changed the Federal Food Drug and Cosmetic Act [7]. Under the Dietary Supplement Health and Education Act of 1994, botanical products (herbals), complementary nutritionals (amino acids, protein – rich foods, etc.) and micronutrients (vitamins, microminerals) are all considered to be dietary supplements [8]. Dietetic products are used up to extend our diet with needed micronutrients, herbs, protein and amino acid for ideal body function [9, 10]. Different types of tea, pills and other similar products are sold to people over the internet for diet products. Diet products can be reached very easily.

Several analytical methods have been used for the determination of phenolic acids in different products: The most commonly published techniques for the phenolic acids are high performance liquid chromatography (HPLC), liquid chromatography–mass spectrometry (LC-MS) and gas chromatography–mass spectrometry (GC-MS) [11-19]. However, in the literature, there are a lot of publications focused on the phenolic acids analysis of tea. In this study, the concentration of some phenolic compounds in dietetic tea products were determined by HPLC-DAD. This study is one of the provincial studies on dietetic teas which are commercially sold on the internet.

MATERIALS and METHODS

Samples

Ten dietetic tea products (of different brands) were purchased from national suppliers, and all samples were in tea powder form.

Standards

Gallic acid, protocatechuic acid, 4-hydroxybenzoic acid, chlorogenic acid, caffeic acid, *p*-coumaric acid, ferulic acid, ellagic acid, epicatechin, rutin and quercetin were purchased from Sigma-Aldrich (Steinheim, Germany) and Merck (Germany). BHT (butylated hydroxytoluene) was supplied by Aldrich (Sigma Aldrich, USA), Methanol (HPLC grade), 37% HCl (ACS reagent) were obtained from Merck (Germany) and Sigma-Aldrich (Steinheim, Germany). All standards were prepared as stock solutions in methanol.

Standard Solutions, Calibration Curves and Recovery Studies

Stock standard solutions of 10 mg/mL of each compound were prepared in methanol and stored at -20°C. In both cases, different working standard solutions were prepared by dilution in the same solvent. The six concentrations used for calibration curves of target compounds. The average recoveries of the analytes were determined by comparing the peak areas obtained from each phenolic acid compounds and extracted from dietetic tea product samples.

Sample Preparation

The extraction method used for dried samples had as follows: 40 mL of 65% aqueous methanol containing BHT (1 g/L) was added to 1.0 g of dried dietetic tea sample. Then the extract was acidified with 10 mL of 6 M HCl under N_2. The extraction mixture was then sonicated for 15 min and was carried out for 2 h at 90°C in a water bath (Termal, J11540KD, Istanbul, Turkey). The mixture was then filtered and made up to 100 mL with methanol [20, 21], furthermore filtered quickly through a 0.45 µm membrane filter membrane filter and injected to HPLC.

The LC Method

The analytical HPLC system employed consisted of a Shimadzu Prominence high performance liquid chromatograph coupled with a 20A CBM (HPLC System Controller), a diode array detector (SPD-M20A, Tokyo, Japan), a SIL 20ACHT automatic sampler, a CTO-10ASVp column oven and a LC20 AT pump. The analytical data were evaluated using a LC Solution data processing system. The separation was achieved on a Agilent ZORBAX Eclipse Plus C18, 4.6 × 250 mm, 5 µm column at 25°C. The mobile phase consisted of water with 3% glacial acetic acid (A) versus (B) methanol. The elution gradient applied at a flow rate of 0.8 mL/min was 95% A/5% B for 3 min, 80%A/20%B in 15 min and isocratic for 2 min, 60%A/40%B in 10 min, 50%A/50%B in 10 min, and 100% B in 10 min until the end of the run. Samples were dissolved in methanol, and 100 µL of this solution was injected into the column [22]. The monitoring wavelength was 280 nm for the phenolic acids and 320 and 370 nm (flavones, flavonoles). The identification of each target compound was based on a combination of retention time and spectral matching.

Statistical Analysis

Limit of detection (LOD), limit of quantification (LOQ), linearity of calibration, and recovery were estimated for the validation of this method. Each phenolic acid compounds concentration was measured in five replicates. We defined the LOD as three times the background noise of the chromatographic instrument. The extraction recovery of this method were determined by spiking blank dietetic tea products with each compound in three replicates; they were extracted as previously described. Recovery was assessed by analyzing the target compounds spiked at 5 different days.

RESULTS and DISCUSSION

HPLC Analysis

Hydrolysis of glycosides or esters to detect a free phenolic compounds content by HPLC-DAD [23]. Sample preparation was applied with a methanol-water extraction. Methanol has an important preservative quality and can prevent the oxidation of phenolic compounds [24]. The retention time (R_t) and wavelength for detection for analyses of phenolic compounds in tea

samples. A summary of all of the phenolic compounds within each class identified in tea samples is given in Table 1.

Analytical Results

When applied to dietary and diet products the proposed method showed good results. The calibration curves for all the species studied showed good linear correlation coefficients ($r^2 \geq 0.999$), independent of the method used for sample preparation (Table 2). The quantitation limit ranged 0.010 to 0.050 µg/g in dietetic tea products (Table 2). Bae et al. [12] evaluated the different extraction methods, and obtained LOD values between 0.06-2.92 mg/L. The LOD 3.50 – 16.80 ng/mL for phenolic compounds were reported in teas [13].

As a result, average recoveries of studied phenolic compounds were higher than 85 % (Table 3).

Table 1. Retention time (R_t) and wavelength (λ_{nm}) for detection for analyses of phenolic compounds

Compounds	R_t (min) (HPLC-DAD)	λ_{nm}
Gallic acid	7.8	280
Protocatechuic acid	12.2	280
4-hydroxybenzoic acid	18.1	280
Chlorogenic acid	19.9	320
Caffeic acid	23.0	280
Epicatechin	29.0	320
p-Coumaric acid	30.3	320
Ferulic acid	35.7	320
Rutin	45.6	360
Ellagic acid	47.7	280
Quercetin	70.4	320

Table 2. Correlation coefficients (r^2), limits of detection (LOD, s/n=3.3), limits of quantification (LOQ, s/n=10) as determined for the methanol-water extraction

Compounds	r^2	LOD (µg/g)	LOQ (µg/g)
Gallic acid	0.999	0.010	0.033
Protocatechuic acid	0.999	0.031	0.102
4-hydroxybenzoic acid	0.999	0.011	0.036
Chlorogenic acid	0.999	0.010	0.363
Caffeic acid	0.999	0.013	0.043
Epicatechin	0.999	0.010	0.033
p-Coumaric acid	0.999	0.014	0.046
Ferulic acid	0.999	0.010	0.033
Rutin	0.999	0.050	0.165
Ellagic acid	0.999	0.045	0.149
Quercetin	0.999	0.048	0.158

Table 3. Methanol-water extraction average recovery (RSD, %)

Compounds	Average Recovery, R % (mean ± SD (%))
Gallic acid	91.43 (1.03)
Protocatechuic acid	95.78 (1.11)
4-hydroxybenzoic acid	90.72 (0.93)
Chlorogenic acid	97.35 (1.21)
Caffeic acid	88.86 (0.97)
Epicatechin	86.78 (1.11)
p-Coumaric acid	89.58 (0.99)
Ferulic acid	93.18 (1.05)
Rutin	95.21 (1.11)
Ellagic acid	86.45 (0.99)
Quercetin	97.11 (1.21)

Bae et al. [12] studied different methods, and recoveries varying from 96.6-103.50%.The recovery values equal and higher than 83% for target compounds were found [13].

The amounts of phenolic compounds detected in the samples are presented in Table 4. 10 samples were analyzed according to the above described method. Results are expressed in mg/100 g dry sample. The most abundant phenolic acids were: gallic acid (ND–6.98 mg/100 g dry sample), protocatechuic acid (0.98–45.61 mg/100 g dry sample), 4-hydroxybenzoic acid (ND–31.56 mg/100 g dry sample), chlorogenic acid (ND–0.73 mg/100 g dry sample), caffeic acid (11.27–154.55 mg/100 g dry sample), *p*-coumaric acid (ND–6.56 mg/100 g dry sample), ferulic acid (ND–1.13

mg/100 g dry sample) and ellagic acid (0.10–1.76 mg/100 g dry sample). Epicatechin (7.59–290.07 mg/100 g dry sample), rutin (ND–49.95 mg/100 g dry sample) and quercetin (1.15–10.50 mg/100 g dry sample) were the main flavonoids identified in diet teas.

Table 4. Phenolic compounds in tea (mg/100 g dw) (mean ± SD (%))

Compounds	1	2	3	4	5	6	7	8	9	10
Gallic acid	4.33 (0.12)	ND	2.85 (0.15)	4.21 (0.10)	0.35 (0.12)	6.98 (0.15)	2.22 (0.16)	3.51 (0.12)	2.70 (0.10)	0.18 (0.12)
Protocatechuic acid	15.22 (0.13)	0.98 (0.18)	8.97 (0.16)	13.45 (0.15)	45.61 (0.19)	29.33 (0.14)	1.52 (0.13)	22.33 (0.14)	1.33 (0.15)	1.07 (0.11)
4-hydroxybenzoic acid	10.30 (0.17)	15.23 (0.15)	3.52 (0.18)	4.78 (0.13)	3.71 (0.17)	13.32 (0.14)	31.56 (0.14)	22.11 (0.15)	ND	8.59 (0.13)
Chlorogenic acid	ND	0.21 (0.10)	0.73 (0.11)	ND	0.29 (0.13)	0.43 (0.12)	ND	0.61 (0.12)	0.18 (0.12)	0.14 (0.14)
Caffeic acid	45.09 (0.18)	33.81 (0.15)	154.55 (0.11)	67.91 (0.13)	22.56 (0.15)	14.44 (0.12)	12.50 (0.18)	33.90 (0.14)	11.27 (0.15)	58.73 (0.13)
Epicatechin	189.71 (0.15)	9.28 (0.17)	21.75 (0.14)	189.43 (0.17)	31.27 (0.18)	11.90 (0.17)	49.95 (0.13)	117.33 (0.11)	290.07 (0.10)	7.59 (0.14)
p-Coumaric acid	2.67 (0.16)	0.31 (0.15)	2.68 (0.16)	0.33 (0.11)	4.59 (0.11)	ND	0.23 (0.13)	6.56 (0.16)	0.10 (0.14)	ND
Ferulic acid	ND	0.92 (0.17)	ND	0.29 (0.19)	1.13 (0.15)	ND	0.83 (0.14)	0.21 (0.18)	ND	0.18 (0.13)
Rutin	11.29 (0.20)	27.41 (0.18)	4.57 (0.21)	8.03 (0.18)	23.21 (0.16)	36.41 (0.21)	49.95 (0.20)	48.31 (0.21)	15.47 (0.18)	ND
Ellagic acid	0.45 (0.21)	0.93 (0.19)	0.97 (0.22)	0.18 (0.20)	1.76 (0.18)	1.67 (0.19)	0.28 (0.21)	0.54 (0.20)	0.10 (0.22)	0.29 (0.21)
Quercetin	8.76 (0.22)	4.31 (0.20)	3.67 (0.24)	5.21 (0.21)	1.98 (0.21)	7.92 (0.22)	10.50 (0.23)	2.23 (0.19)	2.92 (0.20)	1.15 (0.23)

Abbreviations ND; not detected; SD, Standard deviation

CONCLUSIONS

Methanol-water showed high extraction efficiency for dietetic tea products. Average recoveries of target phenolic compounds were higher than 85 %. The LOD ranged 0.010 to 0.050 µg/g in dietetic tea products. The most abundant phenolic acids were: gallic acid, protocatechuic acid, 4-hydroxybenzoic acid, chlorogenic acid, caffeic acid, *p*-coumaric acid, ferulic acid, ellagic acid, epicatechin, rutin and quercetin.

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