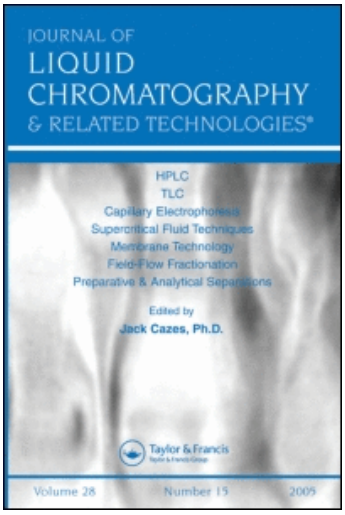

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Significance of Chromatographic and Voltammetric Data for the Classification of Green Teas in Türkiye: A Principle Component Analysis Approach

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Significance of Chromatographic and Voltammetric Data for the Classification of Green Teas in Türkiye: A Principle Component Analysis Approach

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Abstract: Tea (*Camellia sinensis*) is one of the most popular beverages in Anatolia; it provides a significant source of phenolic compounds in the Turkish diet. All around Anatolia, tea traditionally is usually served black but in recent years green tea is becoming a popular alternative, mostly due to its higher content of antioxidant compounds. The growing green tea consumption resulted in the appearance of commercial green tea brands with different additives.

In this study, the flavonoid content of methanol extracts of various commercial green tea samples was determined by HPLC analysis. Rutin, apigenin, luteolin, luteolin-4-glucoside, naringenin, quercetin, isoquercitrine, and quercetin-4-
glucoside were studied as model bioactive flavonoid compounds successfully separated and detected. HPLC detection was performed under isocratic conditions through a C_{18} analytical column with methanol and 0.01 M orthophosphoric acid (pH 7) (50:50, v/v) as the mobile phase. Variable wavelength UV-Vis (230 nm), fluorescence (Ex: 250 nm, Em: 450 nm), and a programmable electrochemical detector (+1.30 V vs. Ag/AgCl) were employed for detection. Based on the chemometric tools (principle component analysis, PCA), the flavonoid patterns of the samples were used successfully to identify and classify the commercial brands with additives from unmodified ones. This might help to make a very easy and rapid classification simply based on rough data without focusing on detailed analysis methods.

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Additionally, cyclic voltammetry (CV) data of the samples was obtained and used again in PCA to identify the commercial brands. Also, based on the CV data, the samples were sorted according to their anodic oxidation potential, which is a measure of the free radical scavenging (antioxidant) capacity of the samples.

Keywords: Classification, Green teas, Principle component analysis

INTRODUCTION

Tea (*Camellia sinensis* (L) O. Kuntze) is one of the most consumed popular non-alcoholic beverages, which provide a significant source of phenolic compounds in Türkiye.[1–3] Traditionally, tea is usually served black in the Turkish diet and black tea is produced mostly in Rize Province on the eastern Black Sea coast, which has a mild climate with high precipitation and fertile soil. On the other hand, in recent years green tea is becoming a popular alternative, mostly due to its higher content of antioxidant compounds. *Camellia sinensis* owns a very rich spectrum of antioxidants belonging to various sub-classes, such as polyphenols, polyphenolic acids, catechins, flavonoids, etc.[4–9]

Recent publications pointed out that flavonoids are one of the major compounds making an important contribution to the antioxidant activity.[10–13] Flavonoids belong to a very rich group of molecules of various chemical structures, thus, under isocratic conditions it would've been very hard to separate such a wide number of molecules simultaneously present in green tea extracts. Although the major flavonoid fraction of green teas is made up of flavan-3-ols, with epigallocatechin gallate being present in the largest amounts, this paper deals with only eight flavonoids (rutin, apigenin, luteolin, luteolin-4-glucoside, naringenin, quercetin, isoquercitrine, and quercetin-4-glucoside), chosen as the model compounds to undergo HPLC analysis. The PCA of instrumental data of catechin and theaflavin fractions of green teas are currently proceeding and will be discussed in a separate paper.

The common property of the entire antioxidants is their unstable chemical character especially when in solution and under heat.[10] Therefore, the industrial procedures during black tea production, treating harvested leaves with heat, may help improve the aromatic taste and the color in solution, but at the same time they are responsible for the dramatic decreases in the antioxidant content.[12] Regarding the green tea production, the oxidation process is stopped after a minimal amount of oxidation by application of heat; either with steam, a traditional Japanese method; or by drying on hot pans, the traditional Chinese method, and the leaves are processed within one to two days of harvesting.

There are various teas which have additives and/or different processing than "pure" varieties. Tea is able to easily receive any aroma, which may cause problems in processing, transportation, or storage of tea, but can be also advantageously used to prepare scented teas. The growing green tea consumption resulted in the appearance of green tea brands with additives such as *Ginkgo biloba*, *Aloe vera*, *Menta piperita*, and *Citrus lemon* in the Turkish tea market. There is no record on the flavonoid patterns of the commercial green tea brands therefore, the availability of a robust method allowing the flavonoid analysis of the entire green tea samples is highly desirable.

Determination of flavonoids and evaluation of their antioxidant activity by electrochemical methods such as cyclic voltammetry (CV) has been performed various times initially,[14–19] but CV data of green teas haven't been used for statistical analysis based on pattern recognition methods, such as principle component analysis (PCA) for the differentiation and grouping of commercial tea brands. Similarly HPLC analyses of flavonoids have been reported various times previously.[20–29] Such approaches usually employed UV-VIS (UVD) or diode-array (DAD) detectors at a time. There are some other detector approaches also being reported,[28,29] to the best of our knowledge, a HPLC method comparing the three different detectors (ECD, UVD, and FLD) simultaneously for flavonoid analysis hasn't been reported yet.

Statistical analysis (PCA) of chromatographic (HPLC) and voltammetric (cyclic voltammetry-CV) have been employed initially for the differentiation and grouping of various plants, their products and foods,[30–35] but not for commercial green tea samples.

Thus, based on the PCA results of both CV and HPLC data, to make a chemotaxonomic identification of commercial tea brands and making an overview on different HPLC detector performances will be a valuable contribution to the literature. Additionally, based on the CV data, the samples will be sorted according to their anodic oxidation potential, which is a measure of oxidizability and thus the CV data will provide useful information about the free radical scavenging (antioxidant) capacity of the samples.

EXPERIMENTAL

Chemicals

Luteolin, naringenin, quercetin-3-glucoside (isoquercitrine), were obtained as standards from Biochemica (Fluka), while quercetin dihydrate, rutin, and apigenin were obtained from Aldrich. These

commercially available flavonoids were donated by Assist. Professor Bintug Ozturk (Ege University, Faculty of Pharmacy). Quercetin-4-glucoside and luteolin-4-glucoside were isolated from the *Helichrysium* species by Professor Ali H. Mericli (Istanbul University/Turkey). They were received from him as a gift and used without further purification. Solvents used for chromatography were methanol and phosphoric acid (HPLC ultragradient grade) supplied by J.T. Baker and Riedel-de Haen AG, respectively. Membranes (0.45 μm pore size) used for filtration of the samples were obtained from Sartorius AG (16555 Minisart©).

Apparatus

The liquid chromatographic system (Agilent 1100 series) supplied by SEM Company (Izmir/Turkey) was equipped with an electrochemical detector (HP 1049-A), a fluorescence detector (G1321A), a variable wavelength UV-VIS detector (G1314A), a pump (G1310A Isocratic Pump), a manual injector (G1328A Rheodyne 7725İ) with 20 μL loop, and a chromatographic data processing software (HP ChemStation for LC Rev. A. 06. 03 [509]).

An additional electrochemical analyzer used for cyclic voltammetry experiments was the Cypress Systems OMNI-101 Microprocessor Controlled Potentiostat with the electrochemical data processing software (Cypress Systems Acquire-101SER, version 1.3.1). Electrochemical experiments were carried out in a 10 mL voltammetry cell (BAS VC-2, part # MF-1052), where Ag/AgCl reference electrode (BAS RE-5B with flexible connector, part # MF-2052) and a platinum wire auxiliary (BAS, 6 cm with gold plated connector, part # MW-4130) joined through the holes in its Teflon cover. The working electrode was a glassy carbon (BAS GCE, 3.0 mm dia., part # MF-2012) and was polished on the microcloth paths (BAS, part # MF-1040) using polishing alumina (BAS, part # CF-1050) before recording each cyclic voltammogram.

Methods

Collection of Commercial Tea Samples

Commercial green tea samples were purchased from various local, well known chain markets in Izmir. Obtained samples represent the majority of all national and imported brands in the Turkish tea market and their information is summarized in Table 1.

Table 1. Brand names and the content of commercial green tea samples collected

Sample no.	Brand name	Content
1	Doğadan	Tea Mint Lemon
2	Doğadan	Tea Ginko biloba
3	Doğadan	Tea Aloe vera
4	Doğadan	Tea
5	Arifoğlu	Tea
6	Botany	Tea
7	Lipton	Tea Aloe vera
8	Beta Tea	Tea
9	Beta Tea	Tea Aloe vera
10	Lipton	Tea Mint

Preparation of Methanol Extracts

Methanol extract preparation was based on a method edited and modified from literature.[36,37] Each green tea sample of 10 g was suspended in 100 mL of 70% methanol and extracted under reflux for 3 hours. The insoluble residue was re-extracted twice with the same solvent and both extracts were combined. The solvent was eliminated by evaporation under vacuum, at 60°C to dryness with a brownish color.

Chromatographic (HPLC) Conditions

The operating conditions were carried out at 24 ± 1°C. Seperation of the model flavonoids was performed under isocratic conditions with a flow rate of 0.5 mL/min for 80 min through an octadecyl (C18) column (Hichrom 5 C18, 7.75×300 mm, 5 μm particle size). The detectors and detection conditions used were as follows; variable wavelength UV-Vis detector (UVD) (λ_{max}: 230 nm), fluorescence detector (FLD) (Ex: 250 nm, Em: 450 nm), and a programmable electrochemical detector (ECD) (E_{App}: + 1.30 V vs. Ag/AgCl). The solvents used and their proportions were as follows : methanol/0.01 M orto-phosphoric acid (50/50 v/v). Both solvents were degassed (ELMA LC 30/H ultrasonic bath) for 30 minutes prior to use. Each compound was tentatively identified by its unique retention time under the same conditions. Quantitative determinations were carried out by the external standard method based on peak heigth or area. The HPLC method described here is a modified version of our previous unpublished validation data.[38]

Table 2. Retention times obtained with electrochemical (ECD), fluorescence (FLD) and UV-Vis (UVD) detectors for various flavonoid standards. (ND* = Not Detectable) Experimental conditions as in *Materials and Methods* section

Flavonoid Standard	t_R (UVD)	t_R (FLD)	t_R (ECD)
Rutin	15.34 min	nd*	15.62 min
Isoquercitrin	18.03 min	nd*	18.34 min
Luteolin-4'-Glucoside	24.71 min	nd*	24.98 min
Quercetin-4'-Glucoside	29.30 min	29.42 min	29.59 min
Quercetin	40.59 min	40.70 min	40.87 min
Naringenin	44.55 min	44.74 min	44.82 min
Luteolin	49.41 min	49.57 min	49.62 min
Apigenin	77.49 min	nd*	77.59 min

HPLC Standard Mixture Preperation

During all chromatographic analysis various standard mixtures were prepared and used. The flavonoid composition of the optimum mixture is as follows:

Isoquercitrin (25 ppm), luteolin-4-glucoside (26 ppm), quercetin-4-glucoside (25 ppm), quercetin (25 ppm), rutin (25 ppm), naringenin (25 ppm), luteolin (45.92 ppm), and Apigenin (45.92 ppm). There were three different retention time (t_R) values for each flavonoid obtained with each detector (UVD, FLD, and ECD), since the detectors were attached in series. Obtained t_R values for each flavonoid compound are displayed in Table 2.

HPLC Sample Preparation

A portion of each dried methanolic extract is weighed properly in the range 30–60 mg (d = 0.1 mg) into disposable Eppendorf tubes and dissolved in 4 mL of methanol (HPLC grade) under sonication for 10 minutes. The prepared sample solution was then filtered using the Sartorius AG (16555 Minisart©) membranes (0.45 μm pore size) and a 100–150 μL portion of the filtered solution was finally injected to the HPLC system using the manuel injector through the 20 μL sample loop.

Cyclic Voltammetry (CV) Conditions

Cyclic voltammograms were recorded in a potential range of 0 to + 1000 mV (vs Ag/AgCl) by a scan rate of 100 mV/sec with the

sensivity of 1 μA/V. During CV experiments, methanol/0.05 M, pH 7.0 phosphate buffer (50/50 v/v with the final pH to be 7.6) was used as the supporting electrolyte.

CV Sample Preparation

A portion (50.0 mg, d = 0.1 mg) of each dried methanolic extract is weighed properly into disposable Eppendorf tubes and dissolved in 2.5 mL of methanol (HPLC grade) under sonication for 10 minutes. The disolved sample was then transferred into the electrochemical cell after filtration, using the Sartorius AG (16555 Minisart©) membranes (0.45 μm pore size) and diluted to 5 mL with 0.05 M phoshate buffer (pH 7.0), with the final pH to be 7.6. CV was then applied to the sample and voltammograms were recorded in text (.TXT) format to be used in PCA.

Data Analysis

HPLC and CV data were analyzed seperately using the chemometric tool called Principle Component Analysis (PCA). With PCA, the experimental data arranged in tables can be reduced to a set of new variables called principle components (PCs). The loadings of the PC is used to define the direction of maximum variability and the score values display the projection of each object onto PC.[30–35] A commercial software, MINITAB® Release v. 14.13 (Minitab Inc.), was used to perform PCA.

Prior to PCA, CV data of all green tea samples were transferred in a MS Excel file to form a matrix. This draft data matrix consists of 11 columns and 172 rows, with the first column to be the potential column where the scanned potential values are displayed. The following columns, each belonging to a commercial tea, contain the data of the first forward scan of the CV.

HPLC data was also saved in text (.TXT) format and each chromatogram was recalled and data was copied again to another MS Excel file to form a matrix. This matrix contains nine columns and ten rows. The first column displayed the sample number of the commercial teas and the following columns display the flavonoid contents for rutin, isoquercitrin, luteolin-4-glucoside, quercetin-4-glucoside, quercetin, naringenin, luteolin, and apigenin, respectively.

These two MS Excel files (one for CV and one for HPLC) are then copied to MINITAB® where data reduction is applied to the draft data matrix of both HPLC and CV, which is a common standardizing procedure simply done by subtracting the mean from each cell in the data column and dividing the resulting new number by the

standard deviation. Since PCA usually focuses on relative differences between objects, with data reduction, each variable (column in the table) is given equal weight in the analysis, which makes the variation the same for all variables. At the end of the data reduction procedure, a new standardized data matrix is formed for each HPLC and CV methods.

PCA was then applied to the standardized data matrix and score plots (for both CV and HPLC) were obtained based on the correlation of the data.

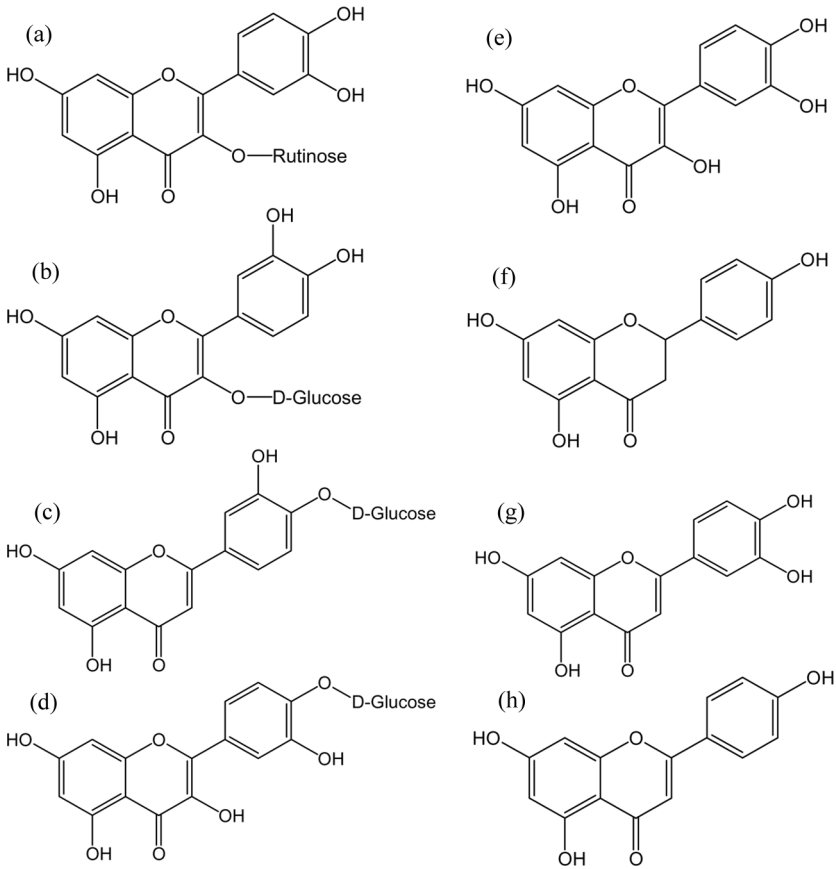


Figure 1. Chemical structures of the model flavonoids studied; Rutin (a), Isoquercitrin (b), Luteolin-4′-glucoside (c), Quercetin-4′-glucoside (d), Quercetin (e), Naringenin (f), Luteolin (g), and Apigenin (h).

RESULTS AND DISCUSSION

The chemical structures of the model bioflavonoids determined by HPLC analysis are summarized in Figure 1. The first four flavonoids are in rutinoside or glycoside forms (A-D), while the remaining is in agluconic form (E-H). These bioflavonoids are determined in ten different commercial green tea samples (Table 1), some of whose ingredients vary due to additives such as mint, lemon, ginkgo biloba or aloe vera. These samples represent the majority of all national and imported brands in the Turkish tea market.

Figure 2 displays the HPLC chromatograms of the flavonoid standards obtained with three different detectors; electrochemical (ECD), fluorescence (FLD), and UV-visible (UVD). As the standard solution, a mixture of flavonoids were prepared as; 25 ppm rutin (A), 25 ppm isoquercitrin (B), 26 ppm luteolin-4-glucoside (C), 25 ppm quercetin-4-glucoside (D), 25 ppm quercetin (E), 25 ppm naringenin (F), 45.92 ppm luteolin (G) and 45.92 ppm apigenin (H). With ECD and UVD all standards could be detected, where as with FLD only four of them (quercetin-4-glucoside, quercetin, naringenin. and luteolin) were detectable. With FLD to detect all entire standards, additional derivatization processes were necessary as most of the flavonoids do not own autofluorescence property. Rutin, quercetin, and luteolin-4′-glucoside is best

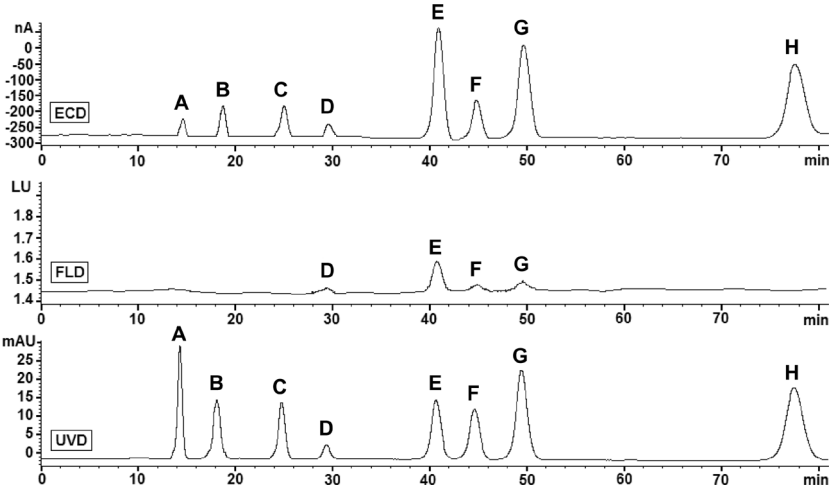


Figure 2. Typical chromatograms of the model flavonoid standards obtained with electrochemical (ECD), fluorescence (FLD), and UV-Vis (UVD) detectors. Experimental conditions as in *Materials and Methods* section and symbols as in Figure 1.

viewed with UVD. Apigenin and naringenin display well defined peaks, where isoquercitrin, luteolin-4-glucoside, and quercetin-4-glucoside show pretty much acceptable responses with ECD & UVD.

The retention time (t_R) values assigned to the model flavonoids are as in Table 2. In accordance to the experimental HPLC conditions, these model flavonoids leave the analytical column in an order of, rutin < isoquercitrin < luteolin-4′-glucoside < quercetin-4′-glucoside < quercetin < naringenin < luteolin < apigenin. Thus, the corresponding retention time values (t_R) are as of 15.34, 18.03, 24.71, 29.30, 40.59, 44.55, 49.41, and finally 77.49 minutes, respectively, with UVD. As the detectors are connected in series there's a slight difference of t_R values for each compound, therefore the t_R values for the remaining two detectors (FLD and ECD) are also displayed in Table 2. Because of detector response characteristics and previously obtained analytical method validation data,[38] UVD is chosen as the optimum detector and data obtained with it is used for the quantification of the model bioflavonoids.

Figure 3, displays a typical chromatogram of commercial green tea samples studied with the three detector (ECD, FLD, and UVD) systems. When all samples are considered, the flavonoid content of this typical chromatogram is replicated for almost all injections. Thus, in qualitative

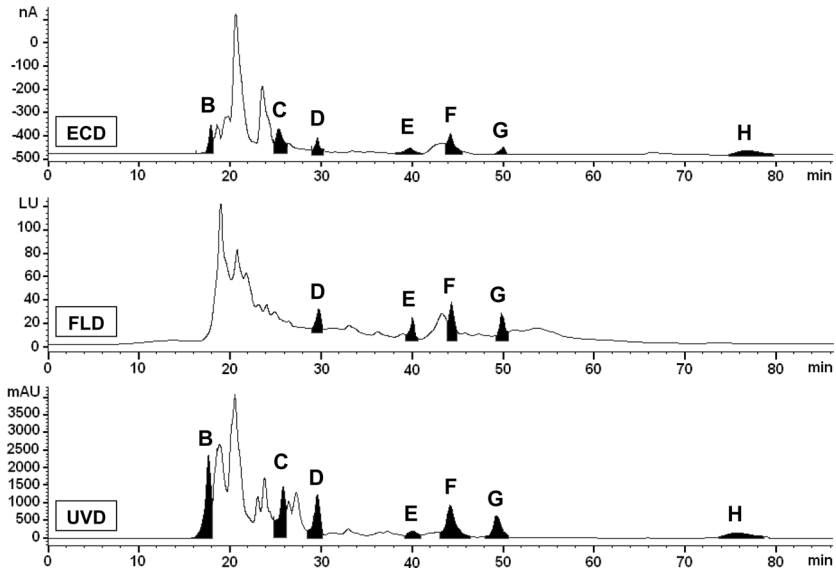


Figure 3. Typical chromatograms of methanol extracts of the green tea samples obtained with electrochemical (ECD), fluorescence (FLD), and UV-Vis (UVD) detectors. Experimental conditions as in *Materials and Methods* section and symbols as in Figure 1.

Table 3. Flavonoid profiles of methanol extracts of various commercial green teas based on HPLC-UVD analysis. Experimental conditions as in *Materials and Methods* section and symbols as in Figure 1

Sample no (commercial brand)	A (ppm)	B (ppm)	C (ppm)	D (ppm)	E (ppm)	F (ppm)	G (ppm)	H (ppm)	Total (A-H)	Anodic (oxidation) peak potential (mV vs Ag/AgCl)
1 (Doğadan)	—	122.62	40.66	11.99	3.06	3.00	—	1.49	182.82	+ 575
2 (Doğadan)	—	124.91	24.64	16.39	3.14	4.65	1.66	1.50	176.89	+ 580
3 (Doğadan)	—	104.49	16.45	13.52	2.55	4.29	1.67	1.55	144.52	+ 600
4 (Doğadan)	—	131.56	28.01	16.66	3.33	4.54	1.79	1.59	187.48	+ 515
5 (Arifoglu)	3.67	126.21	29.47	19.01	2.66	5.79	—	1.52	188.33	+ 500
6 (Botany)	3.69	83.00	30.77	15.51	2.26	5.13	—	1.48	141.84	+ 430
7 (Lipton)	—	134.80	25.20	17.94	3.19	4.04	—	1.51	186.68	+ 505
8 (Beta)	—	94.43	29.58	9.42	2.44	4.03	1.55	1.70	143.15	+ 595
9 (Beta)	—	101.59	43.19	10.99	2.80	3.81	—	1.63	164.01	+ 480
10 (Lipton)	—	117.40	36.39	16.10	3.13	3.73	—	1.61	178.36	+ 430

means, isoquercitrin (B), luteolin-4′-glucoside (C), quercetin-4′-glucoside (D), quercetin (E), naringenin (F), and apigenin (H) are the common flavonoids present, where rutin (A) is a very rare compound only present in two tea samples. Regarding the quantitative means, the amount of each flavonoid varies from sample to sample as expected.

The differences in the flavonoid profiles of the entire samples are summarized in Table 3. As can be seen, the amounts determined by HPLC analysis differs in each sample, while isoquercitrin (B), luteolin-4′-glucoside (C), quercetin-4′-glucoside (D), quercetin (E), naringenin (F), and apigenin (H) are the common flavonoids present in all samples but rutin (A) only present in sample 5 and 6. Isoquercitrin (B) is detected with the highest amount as 134.8 ppm in sample 7 and as 83.0 ppm as the lowest amount in sample 6. Similarly, luteolin-4′-glucoside (C), quercetin-4′-glucoside (D), quercetin (E), naringenin (F), and apigenin (H) are determined in different samples with the highest/lowest amounts to be 43.19/16.45, 19.01/9.42, 3.33/2.26, 5.79/3.0, 1.79/1.55, and 1.7/1.48, respectively. Regarding luteolin (G), only samples 2–4 and 8 shows a similar trend of content at around $\cong$ 1.6 ppm levels, while the compound is not detected in the remaining samples. The total flavonoid content is the highest in sample 5 and lowest in sample 6.

The anodic (oxidation) peak potential values (mV vs. Ag/AgCl reference) of the samples (out of the CV data obtained) are also displayed in the final column of Table 3, which is a measure of oxidizability. When an antioxidant compound has a lower anodic peak potential this implies that the compound is more easily oxidized in comparison to the other antioxidants, or in other words, has a higher antioxidant capacity. Thus, when the anodic peak potentials of the tea samples are compared, the final column provides useful information about the free radical scavenging (antioxidant) capacity of the samples, which is highest in the samples 6 and 10

Table 4. Effects of flavonoids on PCs used in PCA of HPLC data

Variable	PC1	PC2	PC3	PC4	PC5	PC6
Isoguercitrin	−0,462	−0,433	0,140	−0,094	0,529	0,540
Luteolin-4-Gl	0,290	−0,369	−0,746	−0,461	−0,034	0,098
Quercetin-4-Gl	−0,571	0,113	−0,052	−0,252	−0,720	0,277
Quercetin	−0,306	−0,604	0,154	−0,100	−0,099	−0,706
Naringenin	−0,310	0,536	−0,089	−0,578	0,415	−0,320
Apigenin	0,433	−0,109	0,624	−0,609	−0,136	0,148
Eigen value	2,6857	1,8940	0,7386	0,4928	0,1209	0,0680
Proportion	0,448	0,316	0,123	0,082	0,020	0,011
Cumulative	0,448	0,763	0,886	0,969	0,989	1,000
% *Cumulative*	% 44.8	% 76.3	% 88.6	% 96.9	% 98.9	% 100.0

(+430 mV each) and lowest in the samples 3 and 8 (+600 and +595 mV, respectively). There isn't any relationship between the lowest anodic peak potentials and the highest total flavonoid contents. This might still sound acceptable as the antioxidant capacity is not only based on flavonoids but also various other compound groups, such as polyphenols, phenolic acids, procyanidins, anthocyanidins, etc.

The chemometric analysis of the flavonoid contents of the entire samples, determined by HPLC, is performed by principle component analysis (PCA). Table 4 shows that the first three PCs explained more than 88% of the variation of the data set. PC1 was highly connected with the content of quercetin-4′-glucoside and moderately connected with isoquercitrin and apigenin, explaining 44.8% of all the variation. PC2 explained 31.6% of the total variance and was clearly connected with quercetin and naringenin while isoquercitrin content was also important. PC3 is significantly connected with luteolin-4′-glucoside and apigenin while explaining 12.3% of the total variance. PC4 was highly connected with apigenin, naringenin and less with luteolin-4'glucoside, explaining 8.2% of the total variance. PC5 was connected with isoquercitrin and quercetin-4′-glucoside while PC6 was connected with quercetin and isoquercitrin. These two final PCs explained, respectively, 2.0% and 1.1% of the total variance. When the chemical structures of the flavonoids are considered, PC1 and PC5 were connected with gluconic forms (isoquercitrin, quercetin-4′-glucoside) where as PC2 and PC4 with agluconic forms (quercetin, naringenin and apigenin).

Figure 4a displays the loading plot of naringenin, quercetin-4′-glucoside, isoquercitrin, quercetin, luteolin-4′-glucoside, and apigenin for PC1 and PC2. In Table 4 the first two factors, with the highest loadings for PC1 and PC2, are quercetin-4′-glucoside, isoquercitrin, quercetin, and naringenin. In Figure 4a, these compounds are significantly separated from the remaining two (apigenin and luteolin-4′-glucoside) and are concentrated on the left hand side of Figure 4. The Eigen values of all the components are shown by the screen plot in the set (Figure 4a).

In the scatter plot of the score values of the objects projected to PC1 (quercetin-4′-glucoside and isoquercitrin) and PC2 (quercetin and naringenin) plane (Figure 4b), certain groups of green teas can be found, where "P" stands for the plain samples, "Av" for samples with aloe vera, "Mt" for samples with mint and finally "Bt" for the commercial samples "Beta." As figured out from the plot, all plain samples, 4–6 and 8, are concentrated upwards and successfully separated with a reference line from the others. Therefore, the ones beneath the reference line are all modified samples. On the other hand, the modified samples can also be grouped. The samples with aloe vera (Av) (sample 3 and 7) and with mint (Mt) (sample 1 and 10) form two well defined separate groups down in the middle. Interestingly, the plain and modified samples with the

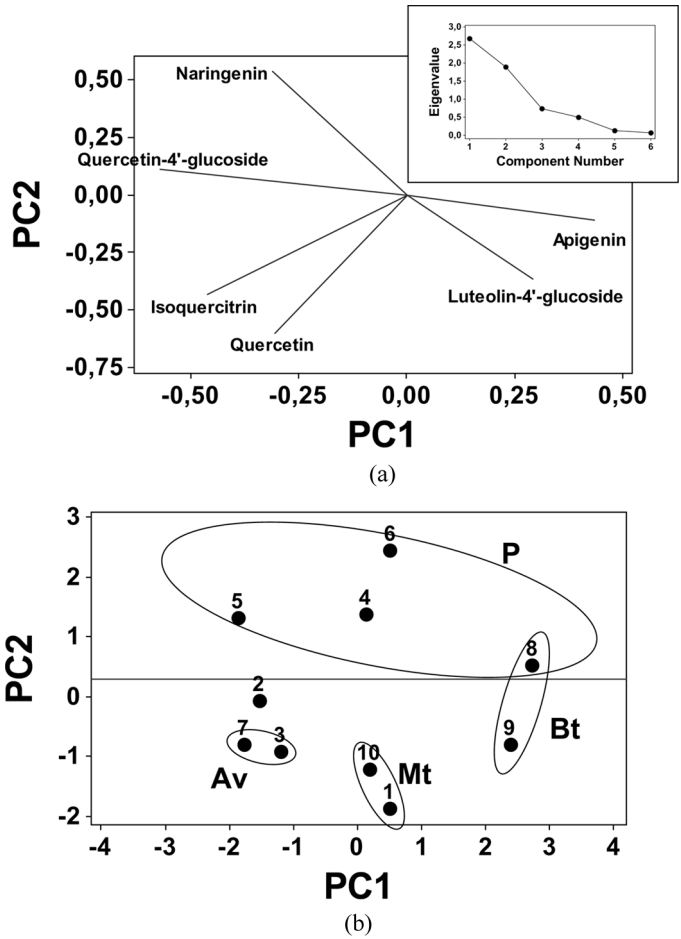


Figure 4. PCA of HPLC data obtained. Loading (a) (Scree plot inset) and Score (b) plots of plain teas (P), teas with Aloe vera (Av), with mint (Mt), and the Beta brand teas (Bt). Experimental conditions as in *Materials and Methods* section.

commercial brand name ''Beta'' (samples 8 and 9) also form another separate group down at the left. The only sample with ginkgo biloba (sample 2) stands near to the samples 3 and 7 with aloe vera (Av). The only possible explanation for this might be that the samples 2 and 3 own the same commercial brand name ''Dogadan,'' which indicates that they might possibly be harvested from the same geographical area and gone through similar production process. Finally, throughout Figure 4, we can say that PCA of the HPLC data (flavonoid contents) is more suitable for the identification of the ingredients of tea samples rather than the identification of their commercial names.

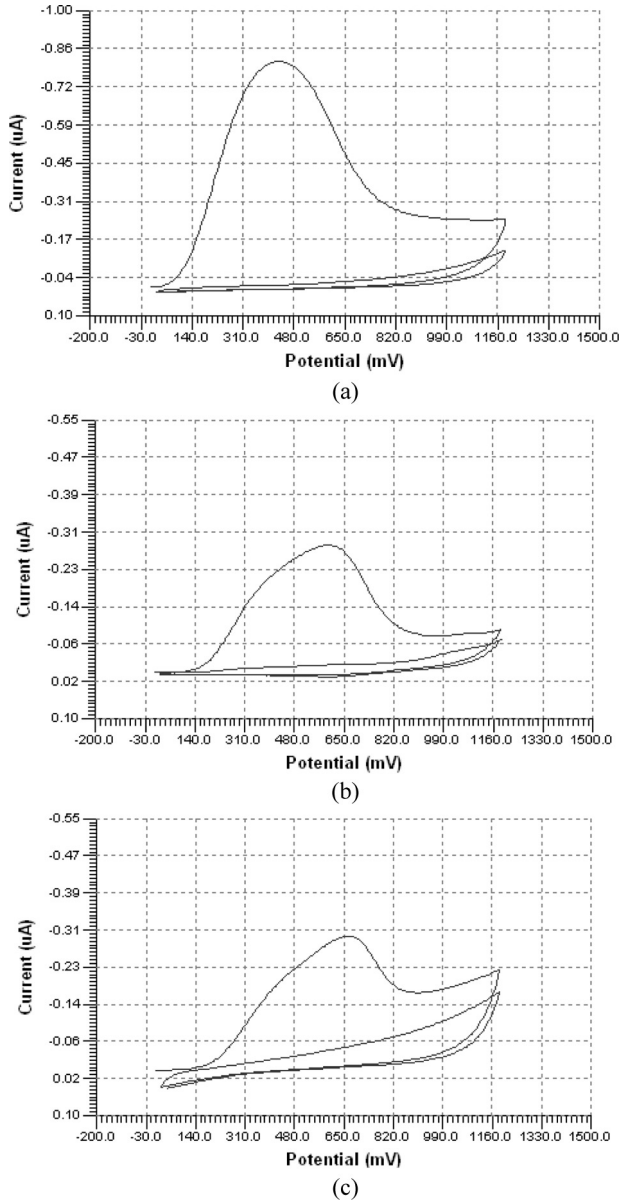
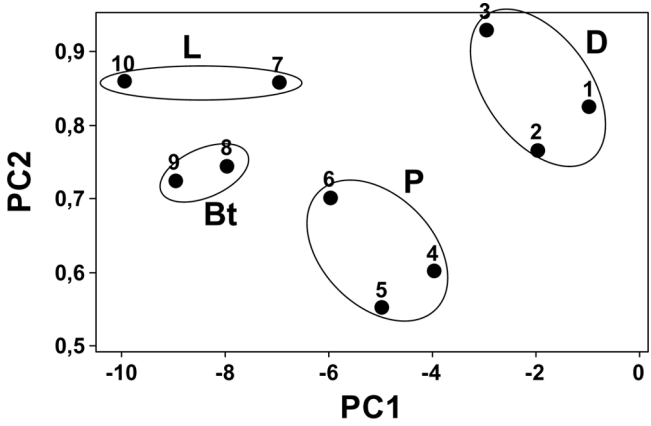
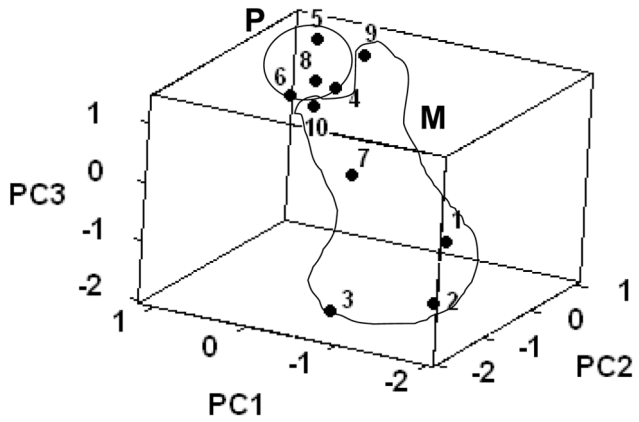


Figure 5. Typical cyclic voltammograms of methanol extracts of green tea samples obtained with GCE (a), AUE (b), and PTE (c). Experimental conditions as in *Materials and Methods* section.

Figure 5 displays the typical cyclic voltammograms (CV) of the tea samples obtained with glassy carbon – GCE (A), gold – AUE (B), and platinum – PTE (C) working electrodes. As can be seen the anodic oxidation peak potential of the first forward scan, differs with the type of working electrode used. Usually the peak potentials are in the order of magnitude GCE < AUE < PTE, which indicates a shift in positive direction. On the other hand, the response currents with different working electrodes also differ, which is the highest with GCE and almost 50% less with AUE and PTE. The peak shapes are also different in each case,



(a)



(b)

Figure 6. PCA of CV data based on two PCs (a) for plain teas (P), Lipton brand (L), Dogadan brand (D), Beta brand (Bt), or based on three PCs (b) for plain (P) and modified (M) teas. Experimental conditions as in *Materials and Methods* section.

which is very well defined with GCE but poorer with AUE and PTE. A similar decaying trend of response current is observed in the following forward scans with all working electrodes. Based on the superiority obtained with GCE to the other electrodes, for PCA only GCE is used.

The score plot of PCA (scatter plot) of CV data of the tea samples are displayed in Figure 6. The score plots based on the first two (Figure 6a) or three (Figure 6b) PCs show interesting results. Figure 6A displays better commercial brand identification than Figure 4B, which identified ingredients better than brands using the HPLC data. The commercial brand names, Lipton (L), Beta (Bt), and Dogadan (D) form well defined separate groups, as can be seen in Figure 6a. The Dogadan group is located upwards at the right hand side, where the Lipton group is located just the opposite, up the left hand side. The Beta group is also located at the left hand side just beneath the Lipton group. Interestingly some remaining plain samples, except sample 8, form another well defined separate group down in the middle. Shortly, it can be said that PCA based on CV data using the first two PCs is more successful for the identification of brand names rather than ingredients.

Interestingly in Figure 6b, when the first three PCs are employed, the score plot of the PCA results in a successful separation of the samples based on ingredients as well. Unlike the detailed separation in Figure 4b, only a rough separation is possible for plain (P) and modified (M) tea samples. This again proves that PCA based on CV data, using either the first two or three PCs, is more successful for the identification of brand names rather than ingredients.

CONCLUSIONS

This study indicates that, even though some similarities can be seen, there are considarable differences in the flavonoid profiles of commercial green teas commonly consumed in Republic of Türkiye.

Although the major flavonoid fraction of green teas is made up of flavan-3-ols, with epigallocatechin gallate being present in the largest amounts, this paper deals with only eight model flavonoids (rutin, apigenin, luteolin, luteolin-4-glucoside, naringenin, quercetin, isoquercitrine, quercetin-4-glucoside) to undergo the HPLC analysis. The PCA of instrumental data of catechin and theaflavin fractions of green teas are currently proceeding and will be discussed in a separate paper.

The results of the HPLC analysis suggest that the highest levels of the model eigth flavonoids are found in commercial samples; Arifoğlu, Doğadan, and Lipton. The difference in flavonoid patterns may successfully be used for grouping tea samples based on commercial brands or

ingredients. Based on chemometric analysis, PCA of the HPLC data is suitable mostly for the classification based on the ingredients of tea samples rather than the identification of their brand names. On the other hand, interestingly PCA based on CV data, using either the first two or three PCs, is more successful for the classification of brand names rather than ingredients.

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