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Determination of Vitamin B2 contents in Black, Green, Sage, and Rosemary Tea Infusions by Capillary Electrophoresis with Laser-Induced Fluorescence Detection

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Abstract: Vitamin B2, also known as riboflavin (RF) is an essential micronutrient for human health and must be obtained from dietary sources. Plants biosynthesize riboflavin and are important dietary sources of vitamin B2 for humans. Our present study reports sensitive detection of Vitamin B2 in widely consumed for tea infusions, namely black, green, sage and rosemary tea infusions, by a capillary electrophoresis method combined with laser induced fluorescence detection. Moreover, the correlation between Vitamin B2 contents of tea plants with their total phenolics (TPs) and antioxidant capacity are evaluated in this study. Whereas green teas have the highest TPs and antioxidant capacity, the highest RF contents are in sage infusions. The RF contents range between 0.34 and 10.36 $\mu\text{g/g}$ for all tea samples studied. Comparing RF contents of tea samples found in this study to the RF contents of known RF sources, tea infusions are proposed as important dietary sources of Vitamin B2.

Keywords: Tea; *Salvia officinalis*; *Rosmarinus officinalis*; Total phenolic; Antioxidant

1. Introduction

Tea from the leaves of the *Camellia sinensis* plant is the most widely consumed drink in the world after water. There are three kinds of tea products produced from the same plant: Black (fermented), green (not fermented) and oolong (partially fermented) teas. Tea is the dietary source of many bioactive compounds. The health benefit effects of tea to cancer and cardiovascular diseases, to obesity and diabetes were extensively reviewed by Khan and Mukhtar [1]. In 2010, world tea production reached over 4.52 million tons [2]. Turkey is the fifth largest producer of tea in the world after China, India, Kenya and Sri Lanka. Furthermore, Turkey has the one of the highest per capita black tea consumption in the world. The attention to green tea in Turkey has been increasing due to its effect against obesity. Sage (*Salvia officinalis*) tea is one of the most popular herbal teas in Turkey. Like black and green teas, sage and rosemary contain many health beneficial bioactive compounds [3,4].

Riboflavin (RF), or its commonly known name Vitamin B2, is a water-soluble vitamin and essential for human health. RF must be obtained from foods since it cannot be synthesized or stored in the body. Vitamin B2 deficiency affects many organs and tissues [5]. Milk, dairy products, meat, fish, dark-green vegetables, and some beverages like beer and wine are important sources of this vitamin. The analysis of RF in food samples is difficult because of the complex matrix of food and very low content of RF in foodstuff. Recently, capillary electrophoresis (CE) has taken great attention in food analysis due to its easy method development availability, low sample consumption, fast analysis times, and inexpensive separation columns [6]. Lately, a combination of laser induced fluorescence (LIF) detector with capillary electrophoresis has provided a remarkable

improvement in detection limits. Since RF has a native fluorescence, RF contents of various foods have been studied by capillary electrophoretic methods using LIF detection [7-12].

Although a significant number of studies have been reported on tea, sage, and rosemary phenolics, almost no information exists concerning the vitamin contents of these plants. Hu and coworkers reported B2 vitamin in two green tea samples [9]. To our knowledge, there is no study on the content of Vitamin B2 in sage and rosemary. The aim of this study is to contribute to the information on the nutritional value of widely consumed tea infusions, by determining the Vitamin B2 contents of tea plants using the CE-LIF method. Moreover, the correlation between B2 contents of tea plants with their total phenolics and antioxidant capacity are evaluated in this study.

2. Materials and Methods

2.1. Materials

Riboflavin, Folin–Ciocalteu reagent, gallic acid, 2,4,6-tripyridyl-s-triazine and $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ were from Sigma Chemical Co (Steinheim, Germany). Di-sodium hydrogen phosphate dehydrates, sodium carbonate anhydrous, sodium acetate trihydrate, and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ were from Merck (Darmstadt, Germany). All solutions were prepared with water purified by an Elga Purelab Option-7-15 model system (Elga, UK).

Four Black (B1-B4) and two green (G1 and G2) tea-bag samples were obtained from local markets of Istanbul as known commercial brands. The B1 sample consists of teas from the East Black Sea region of Turkey. The others are tea blends. According to labels, B2 is a blend of Kenya and Sri Lanka teas. B3 is a blend of Turkish, Kenya, and Indonesia teas. B4 is a blend of Turkey, Sri Lanka, Kenya, and India teas. Two Sage (S1 and S2) and two Rosemary (R1 and R2) dry herb samples were purchased from Istanbul markets as known commercial brands. Two Sage (S3 and S4) and two Rosemary (R3 and R5) dry herb samples were obtained from Boston-USA markets as known commercial brands.

2.2. Method

Separations were performed with an Agilent capillary electrophoresis system (Waldbronn, Germany) equipped with a ZETALIF 2000 LIF detector (Picometrics, Montlaur, France). RF was detected with an excitation at 488 nm and emission at 520 nm by an Ar-ion laser. The data processing was carried out with the Agilent ChemStation software. The separation was performed at 25 kV. The temperature was set at 250 °C. Injections were made at 50 mbar for 6 s. The fused-silica capillary used for separation experiments was 50 μm id and was obtained from Polymicro Technologies (Phoenix, AZ, USA). The total length of the capillary was 67 cm and the length to the detector was 50 cm. The new fused-silica capillary was conditioned prior to use by rinsing with 1 M NaOH for 30 min and with water for 10 min. The capillary was flushed successively by 0.1 M NaOH for 2 min, water for 2 min, and buffer for 5 min at the beginning of every working day and between runs.

2.3. Determination of Total Phenolics (TP)

The total phenolics of each infusion were determined by using the Folin–Ciocalteu method [13]. 300 μl of for each type of infusion was mixed with 1.5 ml of Folin–Ciocalteu's reagent (1:10 diluted with water) and 1.2 ml of sodium carbonate solution (7.5% w/v). The mixture was allowed to stand for 10 minutes at room temperature until a stable color was obtained. The absorbance of 1/10 fold diluted samples was measured by a Shimadzu UV-1800 spectrophotometer at 760 nm. Results were expressed as gallic acid equivalents (GAE) in mg/g. The calibration equation for gallic acid was $y = 49.582x - 0.0185$ ($R^2 = 0.995$).

2.4. FRAP Assay

The ferric-reducing antioxidant powers (FRAP) of the infusions were determined, following the method of Benzie and Strain [14]. The FRAP reagent was prepared containing 1:1:10 ratio of 10 mmol/L 2,4,6-tripyridyl-s-tri-azine (TPTZ) solution in 40 mmol/L HCl, 20 mmol/L FeCl₃ and 0.3 mol/L acetate buffer at pH 3.6, and warmed to 370 C for 10 min. prior to use. The mixture containing 100 µl sample, 100 µl deionized water, and 1.8 ml FRAP reagent incubated at 370 C for 10 min. The absorbance of the 1/10 fold diluted mixture was measured by a Shimadzu UV-1800 spectrophotometer at 593 nm. Results were expressed as µmol Fe²⁺/g. The calibration equation for FeSO₄.7H₂O was $y = 20.044x - 0.0373$ (R²= 0.999).

3. Results and Discussion

3.1. Optimization of separation

Since the pK_a value of RF is 9.69, the molecule gains a negative charge in basic solutions and can migrate under electrical field. Phosphate electrolyte was selected as separation medium. When phosphate concentration was changed between 15 and 75 mM, no significant change was observed in peak shapes. On the other hand, while the pH of separation electrolyte increases, the RF peak separated from the electroosmotic peak (negative water peak) and was easily integrated. The fluorescence intensity of RF was found at maximum at pH 9.9. Finally, 30 mM phosphate at pH 9.9 was selected as the optimal medium for separation and detection.

3.2. Optimization of Extraction

Riboflavin is slightly soluble in water. One g dissolves in 3 - 15 L water, depending on the crystal structure (Sigma-Aldrich Product Information). Riboflavin is heat stable but very sensitive to light [15]. Considering the very small contents of RF in food products, it can be expected that hot water will withdraw the RF in tea leaves. In order to check the stability of RF in hot water vs. time, RF standard solutions in hot water (100 °C) were incubated in a water bath. Riboflavin contents in infusions were determined by the CE-LIF method. Figure 1 shows the comparative results.

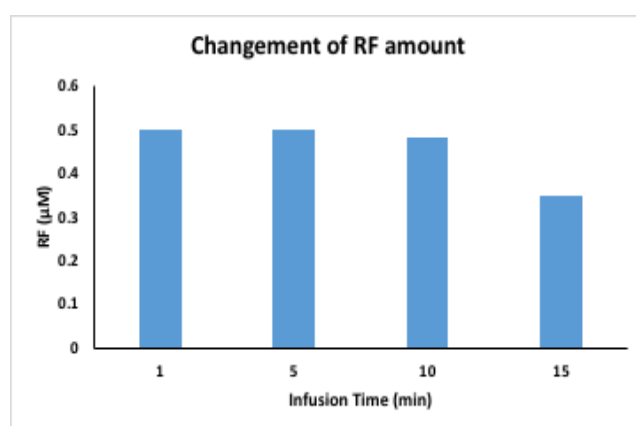


Figure 1. RF amount in hot water vs. infusion time

As seen from Fig. 1, at the end of 5 min. incubation time, we did not observed any decrease of RF concentration in hot water. The decrease of RF content with time after 5 min is probably due to light sensitivity of molecule. Thereby, all tea infusions were obtained in hot water with 5 min. incubation time. 50 mL of boiling water was poured on 1 g of tea leaves and the pot was incubated in a water bath for 5 min. In fact, this is the traditional tea brewing method in homes and coffee houses in Turkey and 5 min is the accepted time to obtain a good tea infusion.

After filtration of tea leaves from hot water, tea infusions were directly injected to the capillary column. Figure 2 shows a representative electropherogram of one sage herbal tea infusion (S3). As seen from the electropherogram, the RF peak comes in less than 4 min. Since infusions were directly injected without any purification or derivatization step, the analysis method of RF is very short and simple.

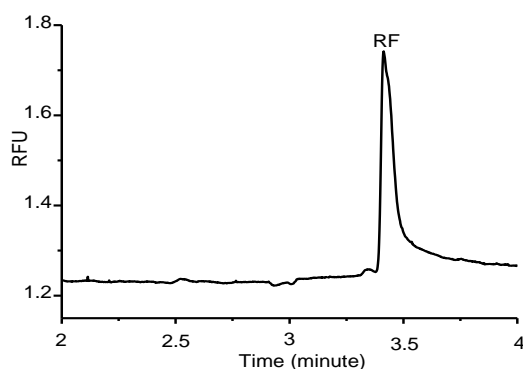


Figure 2. Electropherogram of 1/2 diluted sage herbal tea (S3). Conditions ; 50 μm \times 50 cm capillary , 50 mbar 6s injection, 25 kV running voltage , 30 mM phosphate buffer at pH:9.9

3.3. CE Method Validation

The calibration curve of RF was linear between 0.01-5 μM concentration ranges. The calibration equation was calculated as $y = 0.9544x - 0.0375$ ($R^2 = 0.999$). The limit of detection (LOD) was calculated as 3 times of the average noise taken for three different baseline areas and found as 1.08 ng/mL. The limit of quantification (LOQ) was given as ten times the average noise as 3.58 ng/mL. For the precision of the method, the riboflavin standard solution was injected 5 times in one day. For the day-to-day reproducibility, the same solution was injected five times in three different non-consecutive days. In the same day precision of the corrected peak areas (%RSD) was 2.48%. Between days, the precision value was 4.58 %.

The recovery experiments were done with one herbal tea sample. The infusion was spiked with standard RF solution for three different spike levels at the beginning of the extraction process. Satisfactory recovery for RF was obtained as between 99.7 and 106%.

3.4. Riboflavin Contents of Tea Samples

The RF contents of tea infusions are given as the averages of three infusions with their standard deviations in Table 1.

Table 1. RF content of tea infusions

	<u>RF ($\mu\text{g/g}$)</u>		<u>RF ($\mu\text{g/g}$)</u>
BLACK TEA		GREEN TEA	
B1	3.34 \pm 0.19	G1	3.26 \pm 0.46
B2	0.58 \pm 0.16	G2	2.80 \pm 0.10
B3	1.57 \pm 0.06		
B4	1.07 \pm 0.05		
	<u>RF ($\mu\text{g/g}$)</u>		<u>RF ($\mu\text{g/g}$)</u>
SAGE		ROSEMARY	
S1	5.36 \pm 0.72	R1	2.87 \pm 0.06
S2	5.22 \pm 0.29	R2	0.42 \pm 0.08
S3	6.18 \pm 0.31	R3	1.72 \pm 0.35
S4	10.36 \pm 0.79	R4	0.34 \pm 0.01

As seen from the Table 1, the RF contents change between 0.34 and 10.36 $\mu\text{g/g}$ for all tea samples. Amongst the tested black tea samples, the B1 sample which contains tea leaves from Black Sea region of Turkey was found as having the richest RF content. The RF contents of the blends tested are smaller than this tea. The RF contents of two green tea samples were higher compared the RF contents of black teas. The RF contents of rosemary samples are rather similar to the RF contents in black tea blends. However, the RF contents of all sage teas are substantially higher than those of black and green teas and rosemary infusions. Especially S4 contains significantly higher RF.

3.5. Total Phenolics and Antioxidant Capacities

The TPs and FRAP values of tea infusions are given in Table 2. TPs range from 4.91 to 114 mg GAE/g dry tea leaves for all tea types tested. Green tea samples have the highest TPs compared to both black tea and herbal tea samples.

The FRAP values of the tested teas ranged between 449–492 $\mu\text{mol/g}$ for black tea samples and 552–601 $\mu\text{mol/g}$ for green tea samples. Benzie and Szeto reported FRAP values for 25 types of teas, ranging between 132–654 $\mu\text{mol/g}$ for black teas and 272–1144 $\mu\text{mol/g}$ for green teas [16]. The FRAP values for black and green tea infusions found in this study are in agreement with these reported FRAP values. The antioxidant capacities of sage and rosemary teas range between 63.8–81.9 $\mu\text{mol/g}$ for 8 herbal tea infusions.

TPs and antioxidant capacities of black and green tea infusions are obviously higher than those of herbal tea infusions. As expected, there is a strong correlation (0.985) between TPs and antioxidant capacities of all tea infusions tested in this study. However, there is no correlation between TPs or antioxidant capacity and RF contents of teas. Whereas green teas have the highest TPs and antioxidant capacity, the highest RF contents were determined for sage infusions.

Table 2. The TPs and FRAP values of tea infusions

	RF ($\mu\text{g/g}$)		RF ($\mu\text{g/g}$)
BLACK TEA		GREEN TEA	
B1	3.34 \pm 0.19	G1	3.26 \pm 0.46
B2	0.58 \pm 0.16	G2	2.80 \pm 0.10
B3	1.57 \pm 0.06		
B4	1.07 \pm 0.05		
	RF ($\mu\text{g/g}$)		RF ($\mu\text{g/g}$)
SAGE		ROSEMARY	
S1	5.36 \pm 0.72	R1	2.87 \pm 0.06
S2	5.22 \pm 0.29	R2	0.42 \pm 0.08
S3	6.18 \pm 0.31	R3	1.72 \pm 0.35
S4	10.36 \pm 0.79	R4	0.34 \pm 0.01

Hu and coworkers reported 2.8 and 5.4 $\mu\text{g/g}$ RF for two green tea samples, which is in agreement with our reported values [9]. RF contents of several foods have been reported in the literature. Cataldi et al. have reported RF contents of 8 vegetables ranging between 0.34–1.67 $\mu\text{g/g}$ [7]. The RF contents in five milk samples having different animal origins were reported between 101 and 175 $\mu\text{g}/100\text{ mL}$, in 2 white wine samples as 12 and 13 $\mu\text{g}/100\text{ mL}$, in raw egg white as 3.8 $\mu\text{g/g}$, and in raw egg yolk as 3.2 $\mu\text{g/g}$ [8]. The RF in 12 commercial beers were reported as 13–28 $\mu\text{g}/100\text{ mL}$ [10]. The RF contents of honeys were reported to change in a wide range as from nd to 18.04 $\mu\text{g/g}$ [11]. Riboflavin contents of five saffron samples from two of the biggest producers in the global market (Iran and Spain) were reported in the range of 5.02–13.86 $\mu\text{g/g}$ [12].

Assuming a tea brew obtained from 2 g dry tea (around the mass of dry tea in one tea bag) and 200 mL of hot water (the volume of a tea mug), our reported RF values for dry tea samples as 0.34–10.36 $\mu\text{g/g}$ correspond to the 0.34–10.36 μg RF/100 mL infusion. When RF contents of tea samples found in this study are compared to the riboflavin contents of green vegetables, milk, egg, wine, beer, honey, and saffron samples, known as important RF sources by now, it is seen that tea infusions are also important dietary sources of Vitamin B2.

4. Conclusions

A fast, simple, and sensitive CE-LIF system was used to determine riboflavin (Vitamin B2) contents of 14 tea infusions including black, green, sage, and rosemary teas. The found RF contents of all tea samples suggest that tea infusions are amongst important dietary sources of RF for prevention of diseases caused by Vitamin B2 deficiency.

Author Contributions: Both of two authors designed the experiments; F.T. have done experiments and also calculated-analyzed the data; F.T. wrote the manuscript; F.B.E. supervised the research and wrote and edited the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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