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Determination of Total Antioxidant Capacities as Ascorbic Acid Equivalent of Tea Extract Samples from Different Brands Using Digital Image-Based Colorimetric Detection Method

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Abstract

In this study, the Digital Image-Based Colorimetric Detection Method developed by Bakırdere et al. was used to find the TAC (Total Antioxidant Capacity) value of tea samples from different brands. To determine the total amount of antioxidants in tea samples, the CUPRAC (cupric ion reducing antioxidant capacity) method, which is widely used in antioxidant determination, was combined with a digital image-based colorimetric detection system. To use in our study, a box with opaque wood material measuring 24 cm x 19 cm x 17 cm (width/length/depth) was designed and manufactured. In the analysis, the oxidation reaction between the chromogenic copper(II)-neocuproine (Cu(II)-Nc) reagent and antioxidants was utilized. The color change that occurs as a result of the oxidation was calculated using an application on smartphones. In our study, analyzes were performed on 4 different brand tea extract samples (tea A, tea B, tea C, tea D) to determine the total antioxidant capacity of ascorbic acid equivalent. The TAC values for ascorbic acid equivalent in tea extract samples were found as 380 ± 8 mg/L (tea A), 402 ± 4 mg/L (tea B), 213 ± 3 mg/L (tea C), 232 ± 4 mg/L (tea D) using the digital image-based colorimetric detection systems.

1. INTRODUCTION

The oxygen molecule is a critical molecule for aerobic organisms. It has an important role in the formation and continuation of life. However, the oxygen molecule causes the formation of free radicals in the respiration mechanism and metabolism processes. Free radicals are atoms or molecules with one or more unpaired electrons in their outer orbitals. Free radicals can have a positive or negative charge. They are not stable structures. They are also very reactive despite their short half-life. They can interact very easily inside the cell [1-3].

The main conditions that cause free radical formation in the body can be listed as follows; UV rays, various drugs, fat oxidation, immunological reactions, alcohol, redox reactions, stress, alcohol, and smoking. Free radicals formed in the body are generally hydrogen peroxide, hydroxy radical, superoxide anion, peroxynitrite, hypochlorous acid, and alkoxyl radical [4,5]. Free radicals formed in living metabolism affect the formation and progression rate of diseases such as heart diseases, cancer, diabetes, lung diseases, liver diseases, and aging-related diseases. It is very important to neutralize free radicals in the body to prevent the formation of these diseases or to reduce the rate of progression. Antioxidants play a role in preventing the harmful effects of free radicals [6,7].

Antioxidants in our metabolism are used to prevent the formation of existing free radicals or to eliminate their destructive effects by interacting with existing free radicals. Antioxidants are also the main component of anti-aging products. In addition to being produced by the body, antioxidants can also be taken from the

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outside through food. The main antioxidants taken by food are vitamins (C, A, and E), flavonoids, carotenoids, and polyphenols [8]. Ascorbic acid (vitamin C) is a water-soluble chemical with antioxidant properties. Because of endiol group in its structure, it shows acidic properties as well as reducing properties. It cannot be synthesized by the human body. For this reason, ascorbic acid is taken into the body from the outside through food [9].

Due to the critical role of antioxidants in preventing the extremely negative effects of free radicals in the body, it is necessary to know the total antioxidant capacity (TAC), which reveals the total amount of all antioxidants in the samples. TAC value is one of the criteria used in the processes of determining the quality of foods [10,11].

There are many methods with different scientific approaches to determine the total amount of antioxidants in samples. Total antioxidant capacity (TAC) determination techniques can be grouped under two headings as methods based on electron transfer (ET) and methods based on hydrogen atom transfer (HAT) reactions. While the HAT method is based on competitive kinetic reactions, the color change is used in ET-based methods [12]. The Cupric ion-reducing antioxidant capacity (CUPRAC) method is among the methods of determining antioxidant capacity based on electron transfer [13].

In the CUPRAC method, firstly, copper(II)-neocuproin complex (Cu(II)-Nc) is obtained as a result of the reaction between 2,9-dimethyl-1,10-phenanthroline (Neocuproin-Nc) and Cu(II). The synthesized complex is reduced to copper(I)-neocuproin [Cu(I)-Nc] chelate in the presence of an antioxidant. During this reduction event, the maximum absorbance occurs at 450 nm. Using this approach, the total antioxidant capacity in the samples can be determined [14].

This method is frequently used to determine the TAC values of different antioxidant sources [15,16]. Studies on the determination of antioxidant levels in herbal tea [17], human serum [18], tea [19], and apricot [20] samples by using the CUPRAC technique and using different analysis methods are reported in the literature.

Colorimetric analysis methods developed by utilizing the color change that occurs as a result of the interaction of the analyte with different chemicals in sample analysis have been used for a long time. Recently, with the developing technology, color changes in the sample environment have started to be detected by digital imaging methods [21,22].

The analysis method developed using this imaging technique is called Digital Image Colorimetry (DIC). In digital image colorimetry (DIC), analyses can be performed using RGB (red-green-blue) values in digital images obtained with smartphone cameras, portable cameras, and similar imaging methods. Colors can be evaluated in terms of three basic components (RGB). Each component can be expressed numerically with a number between 0 and 255 [23-26].

In this study, the total antioxidant capacity (TAC) value in tea extract samples belonging to different brands was determined by digital image-based colorimetric detection using the imaging box developed within the scope of the study as the analysis device. The CUPRAC assay was used to determine the TAC value in tea extract samples. The TAC value was calculated as the equivalent antioxidant capacity of ascorbic acid.

2.MATERIALS AND METHODS

2.1. Instrumentation

In the sample preparation procedures, Shimadzu ATX224R analytical balance was used for weighing. pH measurements were conducted with the Ohaus Starter 3100 pH-ion meter. KUDOS SK 3310 HP ultrasonic water bath was used for homogenization in sample preparation processes. In the study carried out, Daihan MSH-20A Magnetic Stirrer with Analog Heater and DLAB MX-S Vortex were employed for mixing processes. A Huawei (Android 9.0) smartphone with 16 megapixels and an f/2.2 lens aperture was used for digital imaging for colorimetric analysis. The flash lamb of the smartphone was not used during digital imaging. To use in our study, a box with opaque wood material measuring 24 cm x 19 cm x 17 cm (width/length/depth) was designed and manufactured (Figure 1). The inner surface of the box is produced in white color to ensure that the light inside is reflected homogeneously in all directions. 12 V white LED strips of equal length are attached to the ceiling and side surfaces of the box. A cutout with a diameter of

2.2 cm was drilled on the cover of the box for the placement of the smartphone's camera. To ensure stability and optimization during the analysis, a sample placement part was made in the box, sized to fit 10 mm quartz cuvettes. The Color Detector smartphone application was used to determine the RGB values during the analysis.

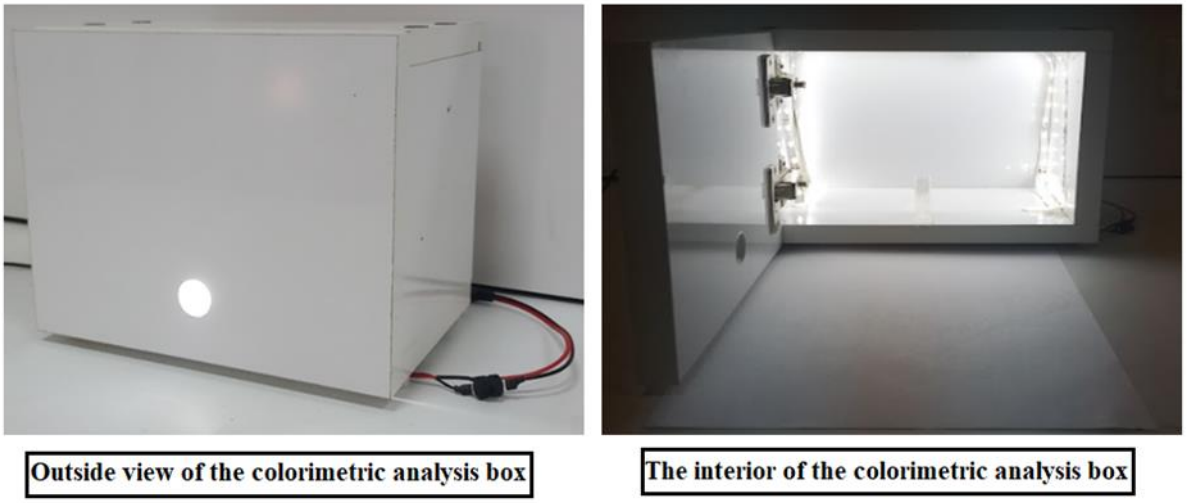

Figure 1. *Schematic representation of the designed colorimetric analysis box*

2.2. Chemicals

Neocuproine standards and Ascorbic acid were obtained from Sigma-Aldrich, Germany. Ethanol was used as a solvent in the stock standard solution to be used during the study for ascorbic acid. The dilution of the standard solution was done with ethanol. As part of the modified CUPRAC analysis, 1.0 x 10^{-2} M Cu(II) solution was prepared by dissolving $CuCl_2$ in deionized water. A standard solution of 9.5 x 10^{-3} M neocuproine (Nc) was obtained using ethanol as solvent. 1.0 M ammonium acetate (NH_4Ac) buffer solution was prepared by using the solution of NH_4Ac in deionized water. NH_4Ac, ammonia solution (25%), CuCl2, and Na_2HPO_4 were all obtained from Merck, Germany. During the study, ultrapure water obtained from the Elektro-Mag Laboratory Water Drop M4 system was used for sample/standard preparation and cleaning of all glassware.

2.3. Digital Image-Based Cuprac Method

In our study, the CUPRAC method described by Bakırdere [23] was used. As part of the CUPRAC method, 1.0 mL of 9.5 × 10^{-3} M solution of neocuproine and 1.0 mL of 1.0 × 10^{-2} M copper(II) were added to a centrifuge tube. Then, 1.0 mL of 1.0 M pH 7.5 ammonium acetate buffer was added to the same tube to adjust the pH of the medium. Finally, 0.4 mL of standard solution was added to the centrifuge tube. The sample volume was made up to 4 mL with deionized water. Colorimetric analysis studies were carried out using the digital imaging box (Figure 1) designed with the last sample obtained. During the analysis, attention was paid to taking the image from the same area in the sample cuvette. Measurements were captured three times for each standard/sample.

2.4. Procedure

Tea extract samples of four different brands were taken from a local market in İstanbul. In the continuation of the study, these teas will be mentioned as tea A, Tea B, Tea C, and Tea D. Using the tea brewing method recommended by the tea companies in their packages, 2 grams of tea sample was added into 125 mL of boiled distilled water. It waited for 15 minutes for the teas to brew. 0.4 mL samples were prepared by diluting the prepared stock tea extract samples 15 times. Then, digital image-based colorimetric analysis of tea extract samples belonging to four different brands was performed. TAC values in the tea samples were calculated by using the results obtained as a result of the analysis and the calibration graph drawn.

3.RESULTS

The values defined by Bakırdere et al. were used as optimum conditions for variables within the scope of the study [23]. Considering the effect of the concentrations of the reagents on the formation reaction of the Cu(II)-Nc complex, 10 mM Cu(II) and 9.5 mM neocuproine were used. Another parameter that affects the complexation reaction is the pH of the medium. A buffer with a pH value of 7.5 was used within the scope of the study. The duration of the oxidation reaction between the synthesized Cu(II)-Nc complex and the antioxidants was determined as 45 minutes. When the R, G, and B color channels used in monitoring the antioxidant concentration in the sample medium are compared, the B channel is used because it has more linear data [23].

3.1. Analytical Figures of MERIT

The analytical performance of the digital image-based colorimetric system based on the CUPRAC assay, which will be used within the scope of our study, was evaluated. For this purpose, colorimetric analysis of samples containing different concentrations of ascorbic acid was performed to obtain a calibration plot. The blue intensity values obtained as a result of the colorimetric analysis of the samples based on digital imaging are shown in Table 1, and the calibration graph drawn using these values is shown in Figure 2.

Table 1. *Blue intensity value of samples at different concentrations*

Concentration (mg/L)	***Blue Intensity***
1	*102*
2	*80*
3	*61*
4	*44*

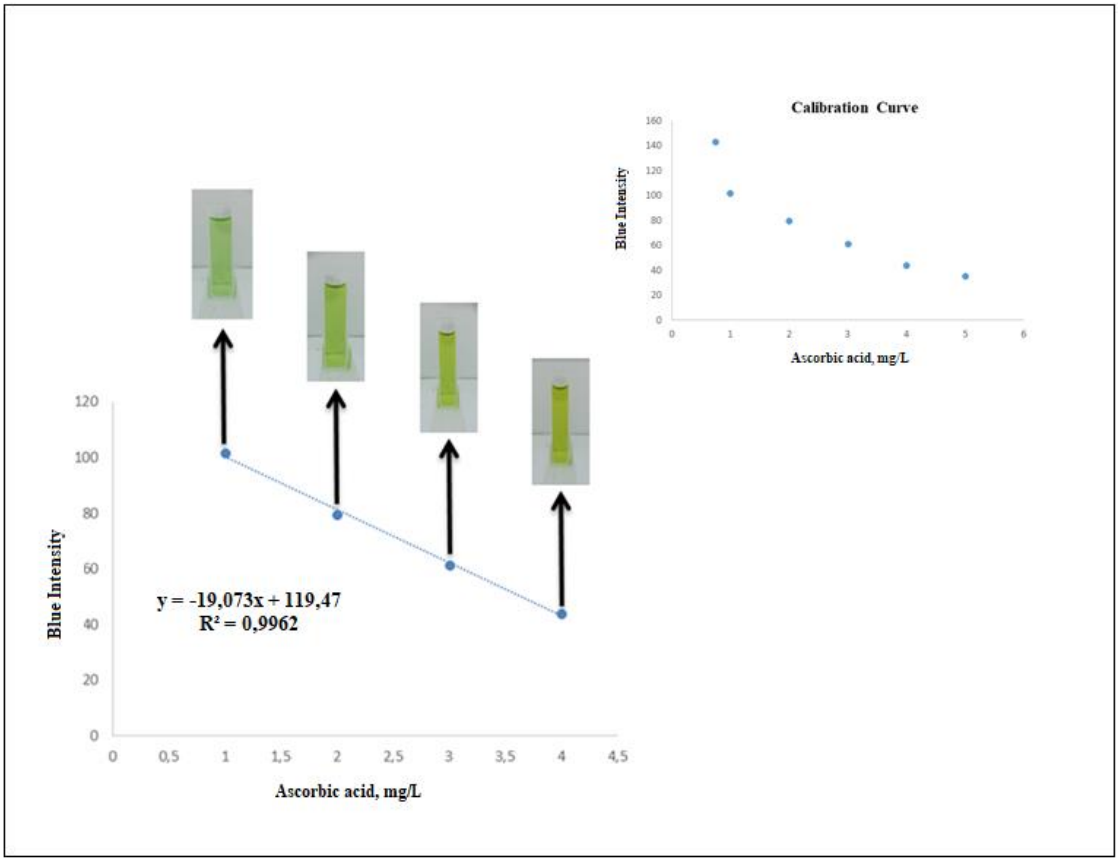

Figure 2. *Calibration plot of varying blue intensity versus increasing concentration of ascorbic acid*

While creating the calibration plot, the intensity of the blue color, one of the RGB color tones, was used as the analysis parameter. When different concentrations of ascorbic acid are added to the Cu(II)/Nc complex, a change in the intensity of the blue color occurs. During the analysis, three digital images were taken for each sample and the color intensity of four different points of each image was measured. As a result of the measurement results, linearity was observed in the range of 1.0-4.0 mg/L. The correlation coefficient of the drawn calibration plot was calculated as 0.9962. Limits of quantification (LOQ) and detection (LOD) values were found to demonstrate the accuracy and sensitivity of the analytical method developed. Eight repetitive absorbance measurements of the lowest concentration in the calibration plot were performed to calculate the LOD and LOQ values. Using these measurement results, the standard deviation (SD) value was calculated. Calculations were made using the 3SD/m (m=slope of the calibration plot) formula for the LOD value and the 10SD/m formula for the LOQ value. As a result of the calculations, the limit of detection value for the analytical method we developed was 0.4 mg/L, and the limit of quantification value was 1.3 mg/L. The percent relative standard deviation value (%RSD) of the analytical method developed was found to be 2.3%. This value shows that the method has high reproducibility.

3.2. TAC Analysis of Different Brands of Tea

Within the scope of TAC analysis, four different brands of tea were purchased. These teas are coded as tea A, tea B, tea C, and tea D. Analyzes were carried out by taking 0.4 mL of the tea extract samples prepared as described in the procedure section. The digital images obtained during the analysis of tea A, tea B, tea C, and tea D are shown in Figure 3.

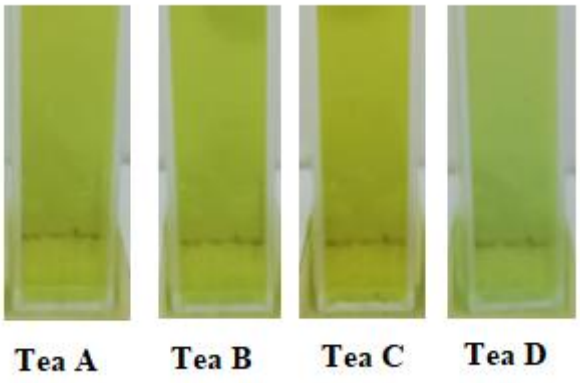

Figure 3. *Digital images of different brands of tea extract samples*

Analyzes based on the blue color intensity of teas belonging to different brands were carried out with digital imaging techniques. Digital-image-based colorimetric analysis results of tea extract samples are given in Table 2.

Table 2. *Colorimetric analysis results of tea extract samples*

Samples	***Blue Intensity***	***TAC Value (mg/L)***
Tea A	*59*	*380 ± 8*
Tea B	*56*	*402 ± 4*
Tea C	*85*	*213 ± 3*
Tea D	*83*	*232 ± 4*

When the results in Table 2 are examined, it is seen that the tea with the highest antioxidant content among the four different tea brands is Tea B. Tea B is followed by Tea A, Tea D, and Tea C, respectively.

4.CONCLUSIONS

In our study, TAC values of teas belonging to different brands were determined by using color change due to the presence of antioxidants during the CUPRAC assay. The color change in the analysis environment was followed by the digital image-based colorimetric method. Within the scope of this study, a completely original digital image-based colorimetric analysis box was designed and manufactured. The developed colorimetric analysis box is made of opaque material. Its inner surface is covered with white-colored material to ensure the homogeneous spread of light around the sample. Analyzes were carried out using a smartphone as the imaging device, thanks to a cutout on the front surface of the colorimetric analysis box. With the developed colorimetric analysis box, firstly, the analysis of ascorbic acid samples with known concentrations was carried out using the CUPRAC assay. A calibration curve was drawn using the measurement results and a linear region was observed in the range of 1-4 ppm ascorbic acid. Antioxidant capacities of tea extracts were calculated as ascorbic acid equivalents using the determined linear region.

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