



# Smartphone Application for Total Phenols Content and Antioxidant Determination in Tomato, Strawberry, and Coffee Employing Digital Imaging

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## Abstract

A low-cost, simple method for in situ determination of total phenols and antioxidants in tomato, strawberry, and coffee through digital imaging was developed and validated. The determination of total phenolic was based on the chelation reaction between quercetin and ferric chloride forming a yellow complex. The antioxidants were quantified by the reaction between 2,6-di-*tert*-butyl-4-methylphenol, potassium ferricyanide, and ferric chloride, forming a bluish-green complex. After optimization, analytical curves were built to monitor the green color channel within the concentration range of 0 to 31.8 mg L<sup>-1</sup> ( $r = 0.9969$ ) and of 0 to 5 mg L<sup>-1</sup> ( $r = 0.9995$ ) for quercetin and 2,6-di-*tert*-butyl-4-methylphenol, respectively. The recovery of quercetin and 2,6-di-*tert*-butyl-4-methylphenol ranged from 96.48 to 101.11% and from 99.00 to 106.00%, respectively. Limits of detection and quantification were 0.03 and 0.11 for quercetin and 0.26 and 0.88 mg L<sup>-1</sup> for 2,6-di-*tert*-butyl-4-methylphenol. The proposed method affords simple manipulation, fast response and environmental safety.

**Keywords** Digital image analysis · Food analysis · Total phenols · Antioxidants · PhotoMetrix® · Method validation

## Introduction

Ingestion of phenolic compounds and antioxidants is important to human health as these compounds control the oxidative stress that causes diseases such as chronic inflammation, cancer, atherosclerosis, diabetes mellitus, and neurodegenerative disorders (Karadag et al. 2009).

Tomato, strawberry, and coffee are among the most consumed foods in the world. Brazil is one of the main producers of strawberry and tomato, with an estimated annual production of 4.5 million tons (IBGE 2017) in addition to being the

largest exporter and the second largest consumer market for coffee in the world (ABIC 2020). Thus, there is a great interest in quantifying phenolic compounds and antioxidants in these foods using a simple, fast, and inexpensive method.

In the literature, several approaches for the determination of phenolic compounds and antioxidants report extraction methods with organic or inorganic solvents followed by high-performance liquid chromatography (HPLC) (Cho et al. 2004; Stalikas 2007; Khoddami et al. 2013), gas chromatography (GC) (Shadkani et al. 2009; Khoddami et al. 2013), or their combinations with mass spectrometry, spectrophotometric assays (Huang et al. 2005; Nacz and Shahidi 2006; Khoddami et al. 2013; Becker et al. 2019), and capillary electrophoresis (CE) (Khoddami et al. 2013). Most of these procedures are time-consuming, laborious, demanding large amounts of reagents, while requiring the use of expensive and sophisticated instruments. Also, the aforementioned classical methods cannot be used for in situ analysis due to their low portability.

To overcome these disadvantages, simple, inexpensive methods that use minimal quantities of reagents employing spectrophotometric techniques associated with image analysis have recently been used to determine different compounds in

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diverse matrices. Common devices such as cameras (Paquetdurand et al. 2012), scanners (Sorouraddin et al. 2015), webcams (Silva Lyra et al. 2009; Santos and Pereira-Filho 2013), and cell phones are used and the images obtained are correlated to a colorimetric and/or reflectance approach that varies according to the concentration of the analyte of interest (Intaravanne et al. 2012).

Benedetti et al. (2015) developed a simple and inexpensive analytical procedure for the determination of sulfite in beverages based on image capture, using the decomposition of system colors, imaged in the primary colors (red, green, and blue), RGB model. A digital flame emission spectrometry (DIB-FES) method for determination of sodium diclofenac in injectable drugs using a webcam to capture the digital image was proposed Lyra et al. (2011). The paper chromatography technique associated with colorimetry was used to determine diversified compounds in food while using a smartphone camera. The contents of the evaluated compounds were obtained by analysis of the images acquired using a software (Monošik et al. 2015). Load coupled device (CCD), sensor, and complementary metal oxide semiconductor (CMOS) cameras, used in analytical chemistry, have rapid signal acquisition, good stability, and linearity, and are suitable for the development of new, fast, and high-quality analytical methods, sensitivity, robustness, and low implementation cost.

These sensors convert the intensity of the incident light into storable digital values capable of generating responses from an image based on the RGB color model, in which three arrays are used to store information (Sena et al. 2011).

Digital images can be decomposed in color using different models, with RGB being the most used (Lopez-molinero et al. 2010). In this model, red (R), green (G), and blue (B), the primary colors, transmit light to display color, and the other colors are generated through a linear combination of these three colors. The generated color intensities are stored in 256 levels on a scale from 0 to 255 for each primary color (R, G, or B), where 0 is pure black (0 for each of the 3 primary colors) and 255 is pure white (mixture of 255 for each primary color) (Paciornik et al. 2006).

In the RGB model, the intensity of the color developed in the chemical reaction is measured as a function of the primary colors that compose it, being directly proportional to the concentration of the analyte. Moreover, the response in RGB channels can be taken as an analytical signal and correlated to the concentration of the analyte of interest (Ravazzi et al. 2018). The signal (S) is obtained differently depending on the imaging program used. In the case of PhotoMetrix®, S is obtained by subtracting the value R, G, or B from the color developed in reaction (I) from the maximum level of color intensity obtained in each R, G, or B (255) primary color, according to Eq. 1.

The combination of digital and colorimetric images or chromogenic methods that can be analyzed from the RGB model is a current possibility for obtaining direct and rapid quantitative determinations (Lopez-molinero et al. 2010). In this work, the digital images were acquired colorimetrically through a reaction between ferric chloride and quercetin, producing a yellow complex, and a reaction between ferric chloride, potassium ferricyanide, and BHT, generating a blue-green complex to determine total phenolic compounds and antioxidants, respectively, by applying the RGB model to the strawberry (*Fragaria* spp. L.), tomato (*Solanum lycopersicum* L.), and coffee (*Coffea arabica* L.). The proposed method is fast, simple, uses small quantities of reagents, and does not require skilled labor, being an economical alternative for food quality control in industrial or artisanal processes in situ.

## Experimental

### Reagents and Samples

Ferric chloride hexahydrate (99%) and trichloroacetic acid ( $\geq 99\%$ ) were purchased from Merck (Wisconsin, USA). Quercetin ( $\geq 95\%$ ), 2,6-di-*tert*-butyl-4-methylphenol (BHT) ( $\geq 99\%$ ), and phosphate buffer 0.2 mol L<sup>-1</sup> were obtained from Sigma-Aldrich (Milwaukee, WI, USA). Potassium ferricyanide ( $\geq 99\%$ ) and ethanol were purchased from Vetec (Rio de Janeiro, Brazil). The purity of all compounds was confirmed by GC analyses.

Four tomato (green–S1, yellow–S2, orange–S3, and red–S4) and three strawberry (green–S5, light red–S6, and dark red–S7) samples, in different ripening stages, were obtained in the local market in Viçosa, Minas Gerais, Brazil (20° 45' 17" S, 42° 52' 57" W).

Seven samples of *Coffea arabica* beans were used, comprising four stages of maturation (green–S8, yellow–S9, orange–S10, and red–S11) and three degrees of roasting (dark–S12, medium–S13, and light–S14). Samples at different ripening stages of maturation (S8–S11) were obtained from crops in Divino de São Lourenço city, in the southeastern state of Espírito Santo, Brazil (20° 37' 12" S, 41° 41' 13" W), while those of at different degrees of roasting (S12–S14) were purchased in the local market in Viçosa, MG, Brazil (20° 45' 17" S, 42° 52' 57" W).

### Solutions

Stock solutions of quercetin (106.0 mg mL<sup>-1</sup>) and of BHT (100.0 mg mL<sup>-1</sup>) in ethanol:ultrapure water (1:1 v/v) were prepared to determine linearity, repeatability, accuracy/recovery, and limits of detection (LOD), as well as quantification

(LOQ) in the total phenols determination and antioxidants, respectively.

A solution of ethanol:ultrapure water (1:1 v/v) has been prepared to extract phenolic compounds and antioxidants from the evaluated foods.

Solutions of potassium ferricyanide 1% (w/v) and trichloroacetic acid 10% (w/v) were prepared in ultrapure water to determine antioxidant activity.

Solution of ferric chloride 1% (w/v) was prepared for the construction of analytical curves, validation of method, and determination of total phenols and antioxidants in the evaluated foods.

To prepare all solutions, the reagents were weighed directly into the vials and the volume completed with the appropriate solvent.

### Sample Preparation

The samples were weighed with an analytical balance from Shimadzu (capacity 10/220 g and accuracy 1 mg/0.1 mg). The volumes were transferred using automatic pipette (Eppendorf, Germany).

Tomato (*Solanum lycopersicum* L.), strawberry (*Fragaria* spp. L.), and coffee (*Coffea arabica* L.) were washed with distilled water and dried at room temperature. Samples of approximately 1 g of these foods were then ground with the aid of porcelain mortar and pestle, then gradually, the mixture of ethanol:water (1:1) was added until reaching the final volume of 50 mL. Extractions were performed in triplicate.

### Apparatus

A single-beam UV-Vis spectrophotometer (Shimadzu, UV Mini 1240) equipped with a 1-cm quartz cuvette was used to perform visible ultraviolet analysis.

A Samsung Galaxy J2 prime cell phone with a resolution of 8.0 MP with the PhotoMetrix® app installed for free from the Google Play Store was used to perform digital image analysis (Helfer et al. 2017).

A wooden box with dimensions of 15.8 cm × 17 cm × 11.5 cm (width × length × height) was made to minimize the entry of external light, with a removable lid to allow the exchange of vials containing sample at each analysis (Fig. 1). A holder was placed on the outside of the case to accommodate the mobile phone and a hole about 2.5 cm in diameter was aligned with the mobile phone's camera and the sample reservoir holder. Another support for fixing the vial of sample/standard solutions, positioned 5.4 cm from the cell phone, was constructed with white ethyl vinyl acetate (EVA) to provide a neutral background for the image to be obtained.

## Determination of Total Phenols Content

### PhotoMetrix® Method

An analytical curve was constructed with quercetin concentrations ranging from 0 to 31.8 ppm ( $n = 7$  points) prepared from quercetin stock solution ( $106 \text{ mg L}^{-1}$ ) in ethanol:ultrapure water 1:1 (v/v) obtained from a Millipore Milli-Q system (USA).

For this 340  $\mu\text{L}$  of quercetin solutions, at different concentrations, was added to 10  $\mu\text{L}$  of ferric chloride solution 1% (w/v). The reading of this mixture was then performed, in triplicate, in the PhotoMetrix® app (Santos et al. 2007) using the green channel at  $\lambda = 500\text{--}580 \text{ nm}$  (Choodum et al. 2013).

The determination of total phenols content in tomato, strawberry, and coffee extracts was also performed by pipetting 340  $\mu\text{L}$  of each extract. Sample preparation, into which 10  $\mu\text{L}$  of 1% (w/v) ferric chloride solution was added. The samples were read in triplicate, using the green channel ( $\lambda = 500\text{--}580 \text{ nm}$ ) (Choodum et al. 2013) from the PhotoMetrix® application (Santos et al. 2007).

### Visible Ultraviolet Spectrophotometry (UV-Vis)

Total phenols content was also determined using the visible ultraviolet spectrophotometry (UV-Vis) technique. To construct the analytical curve, the same quercetin solutions prepared to obtain the analytical curve using the PhotoMetrix® app were used. To obtain the analytical curve and to determine the total phenolic content in tomato, coffee, and strawberry extracts, 2.0 mL of quercetin solutions and extracts were used, respectively, and 70  $\mu\text{L}$  of ferric chloride solution 1% (w/v) were added to each. The reading was taken in triplicate at  $\lambda = 294 \text{ nm}$ .

## Determination of Antioxidant Content (reducing power)

### PhotoMetrix® Method

Determination of antioxidants in tomatoes, strawberries, and coffee was performed using standard 2,6-di-*tert*-butyl-4-methylphenol (BHT), according to the methodology cited by Yen and Chen (1995) with modifications.

Initially, an analytical curve was constructed with different BHT concentrations (0.00, 0.10, 0.50, 1.00, 2.50, 3.50, and 5.00  $\text{mg L}^{-1}$ ;  $n = 7$  points) prepared from stock solution of BHT  $100 \text{ mg L}^{-1}$  in ethanol:ultrapure water 1:1 (v/v). For this, aliquots of the standard BHT solution (0, 10, 25, 50, 75, and 100  $\mu\text{L}$ ) were transferred to seven 25 mL test tubes, to which 2.5 mL of potassium ferricyanide solution 1% (w/v), 2.5 mL of  $0.2 \text{ mol L}^{-1}$  phosphate buffer (pH = 6.6) were added, and the mixture was then incubated at 45 °C for 20 min. Next, 2.0



**Fig. 1** Apparatus made for the determination of phenols and antioxidants in foods. Data collection box: overview front (a), internal view (b), LED cap (c), and photo test tube holder (d), window (e)

mL of trichloroacetic acid 10% (w/v) were added and the volumes were completed to 10.0 mL with ultrapure water.

Three hundred forty microliters of this mixture containing BHT in different concentrations was added to 10  $\mu$ L of 1% ferric chloride solution (w/v) and then read, in triplicate, using the green channel ( $\lambda = 500\text{--}580$  nm) (Choodum et al. 2013) of the PhotoMetrix® application (Santos et al. 2007).

To determine the reducing power in tomato, coffee, and strawberry extracts, a 2.0 mL aliquot of each extract prepared was transferred to a 25-mL test tube to which 2.5 mL of potassium ferricyanide 1% (w/v) and 2.5 mL of phosphate buffer 0.2 mol L<sup>-1</sup> (pH = 6.6) were added. The mixture was incubated at 45 °C for 20 min. Next, 2.0 mL of trichloroacetic acid 10% (w/v) and 1.0 mL of ultrapure water were added to complete the volume to 10.0 mL. The reading using the green channel ( $\lambda = 500\text{--}580$  nm) of the PhotoMetrix® app was performed on 340  $\mu$ L of the mixtures containing extracts, to which 10  $\mu$ L of the 1% (w/v) ferric chloride solution was added. The experiments were carried out in triplicate.

#### Visible Ultraviolet Spectrophotometry (UV-Vis)

As well as the phenolic content, the reducing power was also determined by UV-Vis spectra analysis. The analytical curve was obtained using the same BHT solutions prepared to obtain the analytical curve using PhotoMetrix® app.

The UV-Vis spectra reading was performed at  $\lambda = 706$  nm of both the solutions for obtaining the analytical curve and the mixture containing the tomato, strawberry, and coffee extracts were performed using a volume of 2.0 mL (BHT solutions) and the mixture containing the extracts and 70  $\mu$ L of ferric chloride solution 1% (w/v) (Santos et al. 2007).

#### Comparison Between the Methods Used to Determine Total Phenols and the Reducing Power of Foods

The comparison between the traditional UV-Vis spectrophotometry method and the PhotoMetrix® application used for determination of total phenols and antioxidants in foods was



performed by analysis of variance (ANOVA) and Tukey test with a 95% confidence interval (Cerceanu et al. 2019).

### PhotoMetrix® Validation Method

Validation was performed for the determination of total phenols and for the evaluation of the reducing power of foods. The parameters evaluated were linearity, repeatability, accuracy/precision, and limits of quantification (LOQ) and detection (LOD) (ANVISA 2003; INMETRO 2011; Cerceanu et al. 2016; Franco et al. 2017).

To evaluate the linearity of PhotoMetrix® method for determination of total phenols, a concentration of 0.0, 5.30, 10.60, 15.90, 21.20, 26.50, and 31.80 mg L<sup>-1</sup> of quercetin were added to seven 10 mL volumetric flasks. Volumes of 500, 1000, 1500, 2000, 2500, and 3000 µL containing quercetin (106.0 mg L<sup>-1</sup>) in ethanol:water (1:1 v/v) were added to the volumetric flasks and the final volume was completed to 10 mL with ethanol:water (1:1 v/v) at room temperature. The volume transfers were performed using automatic pipette (Eppendorf, Germany). The experiments were performed in triplicate. For each of the RGB channels, a curve was obtained by plotting a graph using the signal obtained from Eq. (1) vs. concentration of quercetin in different concentrations.

$$S = 255 - I \quad (1)$$

where,  $S$  = signal;  $I$  = R, G or B are the value of the color developed in the reaction, and 255 = maximum level of color intensity obtained in each primary color (R, G, or B).

The mathematical relationship between the signal (response) and the concentration of the analyte of interest was expressed by the analytic curve equation and its correlation coefficient ( $r$ ) (Heleno et al. 2014).

To evaluate the repeatability of the method, the experiments were performed by the same analyst, at the same laboratory, using the same cell phone. The results were expressed as coefficient of variation (CV) obtained by Eq. (2) (Cerceanu et al. 2016).

$$CV = \frac{SD}{\bar{X}} \times 100 \quad (2)$$

where, CV = coefficient of variation;  $\bar{X}$  = average value between the observed concentrations in ten measurements, SD = standard deviation between the observed concentrations in ten measurements.

Accuracy/recovery was determined from recovery tests using the quercetin solutions at concentrations of 5.30, 15.90, and 31.80 mg L<sup>-1</sup>, prepared for the determination of linearity. Ten analyses were performed for each evaluated quercetin concentration. Results were expressed as

percent recovery ( $R$ ), obtained by Eq. (3) (INMETRO 2011).

$$R = \frac{\text{Value}_{\text{observed}}}{\text{Value}_{\text{expected}}} \times 100 \quad (3)$$

The limits of detection (LOD) and quantification (LOQ) were calculated using the relations  $3 \times \sigma/m$  and  $10 \times \sigma/m$ , respectively (Ravazzi et al. 2018; Santos et al. 2019). Where  $\sigma$  is the standard deviation of ten blank measurements and  $m$  is the slope of the analytical curve obtained in determining linearity.

For the validation of the PhotoMetrix® method for determination of antioxidant content in foods (reducing power), 2,6-di-*tert*-butyl-4-methylphenol (BHT) solutions were prepared at the following concentrations: 0.00, 0.10, 0.50, 1.00, 2.50, 3.50, and 5.00 mg L<sup>-1</sup> ( $n = 7$  points), prepared from stock solution of BHT 100 mg L<sup>-1</sup> in ultrapure water:ethanol (1:1 v/v).

The calculations were performed in the same way as for the validation of the method for quercetin determination, and in the evaluation of precision, BHT solutions of 0.1, 1.0, and 5.0 mg L<sup>-1</sup> were used.

## Results and Discussion

In this work, a simple and inexpensive method, which uses digital imaging and a portable device, was employed to determine total phenols and antioxidants in tomatoes and strawberries, at different ripening stages, and in *Coffea arabica* beans at different ripening stages and light, medium, and dark roasting degrees.

For capturing digital images, the built system has been greatly enhanced to ensure the reliability, precision, and reproducibility of the obtained RGB data. First, the best position for fixing the LED lamp plate located on the upper wall of the compartment was evaluated, as well as the intensity of the light emitted to improve the image capture. It is common to find shadows that mainly affect the repeatability of the DIB method evaluated by the relative standard deviation. Thus, better placement of the dividing plate within the chamber is performed to reduce shadows or excessive reflections on the sample and on other regions to avoid erroneous results.

### Construction of the analytical curve for determination of total phenol content

Determination of total phenol content in the evaluated foods was based on the chelation reaction between quercetin and ferric chloride forming a yellow complex with absorption at  $\lambda = 294$  nm.

Initially, an analytical curve for each RGB channel (Fig. S1) was obtained by plotting the  $x$ -axis as the quercetin concentration ( $\text{mg L}^{-1}$ ) and the  $y$ -axis as the signal ( $S$ ), by which  $S$  is obtained from Eq. (1).

Evaluating the analytical curves obtained in the R ( $y = 1.9879x + 47.821$   $r = 0.9528$ ) G (green  $y = 1.5929x + 58.964$   $r = 0.9969$ ) B ( $y = 2.1214x + 59.93$   $r = 0.9819$ ) channels (Fig. S1), it is possible to notice that although B and R channels are very sensitive to quercetin concentration variations (they have high angular coefficient, 2.1214 and 1.9879, respectively), the analytical curves obtained from them are nonlinear since it has correlation coefficient ( $r$ ) less than 0.99 (ANVISA 2003).

The analytical curve obtained in channel G is linear ( $r = 0.9969$ ) and has good sensitivity (angular coefficient = 1.5929), although it is less sensitive than those obtained in channels R and B. Thus, the analytical curve obtained in channel G, with a wavelength in the range from 500 to 580 nm, was used to determine the total phenols in tomatoes, strawberry, and coffee extracts.

An analytical curve for the determination of total phenols by the traditional UV-Vis spectrophotometry method was constructed using the same quercetin solutions as the curves of Fig. S2. The equation of the analytical curve ( $y = 0.0362x + 0.6395$   $r = 0.9991$ ) was obtained by using the  $x$ -axis quercetin concentration ( $\text{mg L}^{-1}$ ) and the  $y$ -axis the UV-Vis absorbance at  $\lambda = 294$  nm (Fig. S2).

### Construction of the analytical curve for determination of antioxidants (reducing power)

The determination of reducing power of tomato, strawberry, and coffee was evaluated based on the reaction between ferric chloride ( $\text{FeCl}_3$ ), potassium ferricyanide ( $\text{K}_3[\text{Fe}(\text{CN})_6]$ ), and 2,6-di-*tert*-butyl-4-methylphenol (BHT), generating a bluish green complex with absorption at  $\lambda = 706$  nm. The color intensity developed in the chemical reaction (complex formed) is directly proportional to the concentration of the analyte (antioxidants) present in the evaluated food.

Just as in determining total phenols, analytical curves for antioxidant determination employing PhotoMetrix® method were built using the R ( $y = 37.6 \times + 63.606$   $r = 0.9944$ ), G ( $y = 37.16 \times + 57.397$   $r = 0.9995$ ), and B ( $y = 15.494 \times + 82.397$   $r = 0.9929$ ) channels (Fig. S3).

According to National Health Surveillance Agency (ANVISA 2003), the analytical curves obtained in the RGB channels are linear since they present  $r$  greater than 0.99 (Fig. S3). The sensitivity of R and G channels to BHT concentration variation (37.6 and 37.16, respectively) is about the same and twice that of the B channel (15.494). Because the analytical curve obtained in channel G ( $\lambda = 500$ – $580$  nm) is the most linear ( $r = 0.9995$ ) and sensitive to BHT concentrations, it was

used to determine antioxidants in tomato, strawberry, and coffee extracts.

The analytical curve ( $y = 0.2995x + 0.1013$   $r = 0.9956$ ) for antioxidant determination by UV-Vis spectrophotometry is shown in Fig. S4. It was built by plotting a graph with the  $x$ -axis BHT concentration ( $\text{mg L}^{-1}$ ) and the  $y$ -axis absorbance obtained from UV-Vis at  $\lambda = 706$  nm using the same BHT solutions prepared for the analytical curve construction using the PhotoMetrix® application.

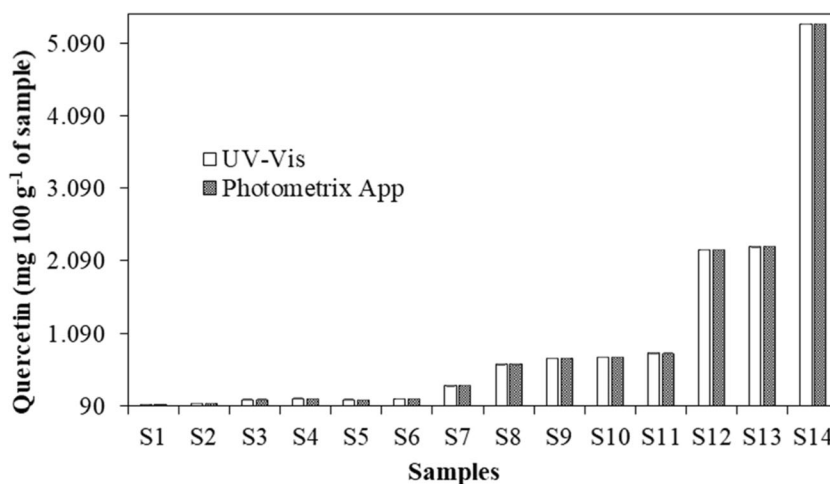
### Determination of Total Phenols and Antioxidants in Foods

With the analytical curves constructed, the content (100 mg of analyte  $100 \text{ g}^{-1}$  of food) of total phenols and antioxidants obtained by using the PhotoMetrix® App in tomatoes, strawberries, and coffee at different stages of ripeness and different degrees of roasting for coffee, were compared to those obtained by visible ultraviolet spectrometry method (UV-Vis) (Figs. 2 and 3). In the determination of total phenols using the Photometrix App, the standard deviation varied from 0.11 to  $4.38 \text{ mg } 100 \text{ g}^{-1}$  in green (S1) and oranges (S3) tomatoes, respectively (Table S9). When UV-Vis was used, the standard deviation variation was  $1.16$  to  $7.60 \text{ mg } 100 \text{ g}^{-1}$  in yellow coffees (S8) and green strawberries (S4), respectively (Table S9). In the evaluation of antioxidant content using the Photometrix App, the standard deviation ranged from 0.05 to  $3.29 \text{ mg } 100 \text{ g}^{-1}$  in green strawberries (S5) and dark roasted coffee (S12), respectively (Table S10). Using UV-Vis method was used, the variation of the standard deviation was from 0.01 to  $5.57 \text{ mg } 100 \text{ g}^{-1}$  in green strawberries (S4) and in dark roasted coffee (S12), respectively (Table S10).

As seen in Fig. 2, the content of total phenols varies with the degree of maturation of the foods. These results are in agreement with Bravo (1998), who states that the amount of phenolic compounds present in foods is strongly associated with genetic factors and environmental conditions, such as the degree of maturation, cultivars, and others. Although the total phenolic content in red tomatoes (S4)  $183.67 \pm 3.01 \text{ mg } 100 \text{ g}^{-1}$  (see Supplementary Information, Table S9) did not vary much when compared to orange tomatoes (S3)  $172.10 \pm 4.38 \text{ mg } 100 \text{ g}^{-1}$  (see Supplementary Information, Table S9), a small difference is observed in relation to yellow tomatoes (S2)  $118.9 \pm 1.970 \text{ mg } 100 \text{ g}^{-1}$  (see Supplementary Information, Table S9). In addition, the percentage of total phenols in red tomatoes is almost double that found in green tomatoes (S1)  $93.72 \pm 1.87 \text{ mg } 100 \text{ g}^{-1}$  of sample (see Supplementary Information, Table S9).

The total phenols content found in dark red strawberry (S7)  $362.5 \pm 1.97 \text{ mg } 100 \text{ g}^{-1}$ ; (see Supplementary Information, Table S9) is twice as high as in light red strawberries (S6)  $176.53 \pm 1.53 \text{ mg } 100 \text{ g}^{-1}$  (see Supplementary Information, Table S9). However, no significant difference was observed

**Fig. 2** Content (mg 100 g<sup>-1</sup>) of total phenol (quercetin) in the food samples of green tomato (S1), yellow tomato (S2), orange tomato (S3), red tomato (S4), green strawberry (S5), light red strawberry (S6), dark red strawberry (S7), green coffee (S8), yellow coffee (S9), orange coffee (S10), red coffee (S11), dark roasted coffee (S12), medium roasted coffee (S13), light roasted coffee (S14) measured by visible ultraviolet spectrophotometry (UV-Vis), and PhotoMetrix® App methods



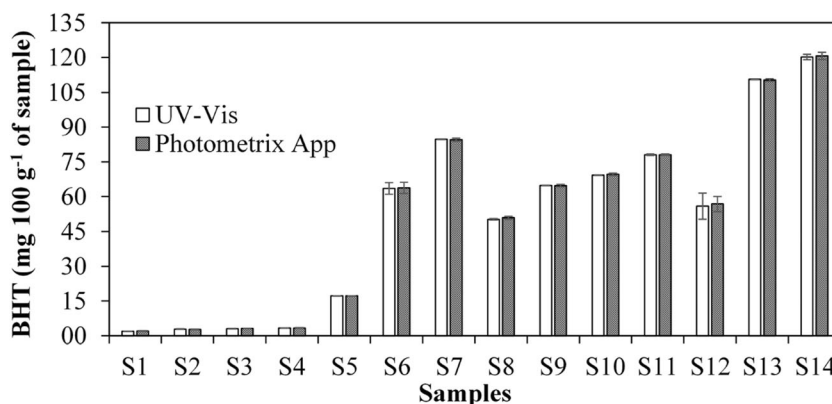
when the light red strawberry (S6) was compared to the green fruit (S5)  $168.29 \pm 0.72$  mg 100 g<sup>-1</sup> (see Supplementary Information, Table S9). The phenols content found in light red and green strawberries agrees with that described by Bravo (1998), between 218 mg per 100 g of fresh material. Although, the phenolic content found in the dark red strawberry (S7)  $362.50 \pm 1.38$  mg per 100 g of fresh, dark red strawberry was higher than that reported by Bravo (1998).

As can be seen in Fig. 2, the maturation stage does not have a great influence on the phenols content in coffee, since the green (S8), yellow (S9), orange (S10), and red (S11) beans showed no significant difference in relation to the phenols percentage  $655.92 \pm 1.05$ ,  $738.07 \pm 1.20$ ,  $759.76 \pm 1.40$ ,  $810.71 \pm 1.74$  mg per 100 g for red, orange, yellow, and green coffee, respectively (see Supplementary Information, Table S9). Moreover, it was observed that the highest total phenolic content is found in coffee submitted to the light roasting process S14  $5358.99 \pm 1.03$  mg 100 g<sup>-1</sup> of phenolic compounds in 100 mg of coffee (see Supplementary Information, Table S9). As the degree of roasting increases, the phenolic content decreases gradually, as observed in

Supplementary Information, Table S9;  $358.99 \pm 1.03$ ,  $2280.06 \pm 1.14$ , and  $2243.32 \pm 1.18$  mg of quercetin per 100 g of coffee subjected light (S14), medium (S13), and dark (S12) roasting, respectively. These results show that the roasting process has a direct influence on the chemical composition of coffee.

Studies report that the quality of the coffee drink has been affected by the roasting procedure, changing the chemical composition of the grains and their antioxidant compounds. This is because during this process, transformations of different chemical constituents occur (Narita and Inouye 2011), in addition to the degradation of chlorogenic acid (Clifford 2000).

The antioxidant content was evaluated at different ripening stages in tomato, strawberry, and coffee. Additionally, different coffee roasting degrees were also evaluated (Fig. 3). It was observed that the amount of antioxidants found in red tomato (S4)  $3.33 \pm 0.12$  mg per 100 g; (Supplementary Information, Table S10) is similar to that found in orange tomato (S3)  $3.10 \pm 0.13$  mg per 100 g; (Supplementary Information, Table S10) and that the antioxidant content decreases in the yellow (S2)



**Fig. 3** Content (mg 100 g<sup>-1</sup>) of antioxidants (BHT) in the food samples of green tomato (S1), yellow tomato (S2), orange tomato (S3), red tomato (S4), green strawberry (S5), light red strawberry (S6), dark red strawberry (S7), green coffee (S8), yellow coffee (S9), orange coffee (S10), red

coffee (S11), dark roasted coffee (S12), medium roasted coffee (S13), light roasted coffee (S14) measured by visible ultraviolet spectrophotometry (UV-Vis) and PhotoMetrix® App methods

and green tomatoes (S1)  $2.72 \pm 0.15$  and  $2.00 \pm 0.14$  mg per 100 g, respectively; (Supplementary Information, Table S10).

The dark red strawberry (S7)  $84.57 \pm 0.68$  mg per 100 g; (Supplementary Information, Table S10) has the greatest reducing power when compared to other foods at the same maturation stage  $3.33 \pm 0.12$  and  $78.09 \pm 0.26$  mg per 100 g for red strawberry (S4) and red coffee (S11), respectively; (Supplementary Information, Table S10). However, the reducing power of strawberries decrease considerably in the other stages of ripening  $84.57 \pm 0.68$ ,  $63.73 \pm 2.43$ ,  $17.24 \pm 0.05$  mg per 100 g for dark red (S7), S6 light red (S6), and green (S5) strawberries, respectively; (Supplementary Information, Table S10).

The results obtained agree with studies by Melo et al. (2008), who found that the antioxidant capacity is influenced by fruit type (species and varieties), stage of maturation, and crop conditions.

Although there is no significant difference in antioxidant content in the different stages of coffee maturation, the red beans presented greater reducing power when compared to the other maturation stages  $78.09 \pm 0.26$ ,  $69.61 \pm 0.57$ ,  $64.73 \pm 0.52$ , and  $50.99 \pm 0.64$  mg per 100 g, for red (S11), orange (S10), yellow, and green coffee (S9), respectively; (Supplementary Information, Table S10).

Studies report that coffee quality has been affected by the roasting procedure which alters the chemical composition of beans, including their antioxidant compounds, because during this process, transformations of different chemical constituents occur (Narita and Inouye 2011), in addition to the degradation of chlorogenic acid (Clifford 2000).

The relationship between the roasting degrees of the coffee and the antioxidant capacity varies according to the species and roasting conditions (Schwarz et al. 2001). According to Narita and Inouye (2011), phenolic acids are progressively destroyed and transformed during the roasting process, resulting in products which may exhibit antioxidant activity.

These results corroborate the work of Trugo and Macrae (1984), who claim that this is probably due to the different phenolic contents present in light and dark roasted coffee and unroasted green coffee beans.

In fact, the results indicate that the roasting degree has a direct influence on the antioxidant content of coffee (Fig. 3). The antioxidant content found in light roasted coffee (S14)  $120.72 \pm 1.56$  mg per 100 g (Supplementary Information, Table S10) was twice as high as that found in dark roasted coffee (S12) and green unroasted beans (S8)  $56.80 \pm 3.29$  and  $50.99 \pm 0.64$  mg per 100 g, respectively (Supplementary Information, Table S10).

The results obtained agree with those reported by Santos et al. (2007), who also observed that coffee samples with a clear roasting degree presented the greatest reducing power. Other researchers also reported that the antioxidant capacity of

the beverage prepared with roasting the coffee beans was double that of green unroasted beans (Stalmach et al. 2006).

The results obtained in this present study show that due to the high amount of antioxidants contained in tomato, strawberry, and coffee, the intake of these foods is beneficial to human health. This is because antioxidants are capable of capturing free radicals in the human body, preventing diseases caused by cellular damage resulting from these radicals, and improving the immune system.

## Comparison Between Methodologies

Statistical tests using ANOVA and Tukey's test proved the similarity of the methods (PhotoMetrix® App and UV-Vis) used to quantify total phenolic compounds and antioxidants for all evaluated foods. Thus, the developed method stands out as a viable alternative to determine total phenolic compounds and antioxidants in plant species, which is useful in laboratories with limited financial and analytical resources in addition to using techniques that corroborate with Green Chemistry.

## PhotoMetrix® App Validation Method

The parameters linearity, repeatability, accuracy/recovery, limit of quantification (LOQ), and limit of detection (LOD) were evaluated for both total phenolic compound and antioxidant determinations (ANVISA 2003; INMETRO 2011).

To evaluate the linearity of the method, the equations of the analytical curves were obtained in the green channel, with seven points, by linear regression ( $y = 1.5929x + 58.964$ ) in the range 0 to 31.8 mg L<sup>-1</sup> and ( $y = 37.16x + 57.397$ ) in the range 0 to 5 mg L<sup>-1</sup> for quercetin and BHT, respectively. For all results, the coefficient of correlation obtained was higher than the minimum acceptance criteria described by Guide on Validation of Chemical Testing Methods (INMETRO 2011) and by Resolution RE 899 – Guide for Validation of Analytical and Bioanalytical Methods (ANVISA 2003), ( $r \geq 0.99$ ) (Figs. S1 and S3).

According to the results presented in Tables S2 and S6, the PhotoMetrix® method was considered repeatable since the values of coefficient of variation obtained (CV) were lower than the maximum permitted by RE 899 ( $CV \leq 5.0$ ) (ANVISA 2003).

Quercetin recoveries were  $101.11 \pm 6.24\%$  (5.30 mg L<sup>-1</sup>),  $96.48 \pm 2.04\%$  (15.9 mg L<sup>-1</sup>), and  $99.37 \pm 1.33\%$  (31.8 mg L<sup>-1</sup>), as noted in Tables S3.1, 3.2, and 3.3, respectively. In the case of BHT, the recoveries were of  $106.00 \pm 9.66\%$  (0.10 mg L<sup>-1</sup>),  $99.00 \pm 1.83\%$  (1.00 mg L<sup>-1</sup>), and  $99.25 \pm 0.45\%$  (5.00 mg L<sup>-1</sup>), as noted in Supplementary Information, Tables S7.1, 7.2, and 7.3, respectively. Since RE-899 sets no limits for the accuracy of the method, this parameter was assessed according to the Brazilian Association of Technical Standards recommendations NBR14029 (ABNT 2005). According to this standard, the analytical procedure must be



able to recover between 98 and 102%. Since most of the values obtained are within or near this range, it can be concluded that the recovery for total phenolic compounds (quercetin) and antioxidants (BHT) is adequate.

The limits of detection (LOD) and quantification (LOQ) of the method estimated by the analytical curve's parameters were 0.03 and 0.11 mg L<sup>-1</sup> (Table S4) for total phenolic compounds, and 0.26 and 0.88 mg L<sup>-1</sup> (Supplementary Information, Table S8) for antioxidants. These values show that the method is able to detect and quantify total phenolic compounds and antioxidants present in food in small quantities.

## Conclusions

The digital image method developed using a smartphone is adequate for determination of total in situ phenolic compounds and antioxidants in tomato, strawberry, and coffee, with good linearity, repeatability, accuracy/recovery, precision, and low detection and quantification limits.

Therefore, the method developed is suitable for determination of total phenolic compounds and antioxidants in foods, with results comparable to those obtained by visible ultraviolet spectrometry, but with the advantage of being simple, low-cost, easy to operate, portable, fast, and environmentally friendly in addition to being accessible to small producers and family agriculture businesses as well as laboratories with limited resource Supplementary Information

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**Authors' Contributions** All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Edmar J. O. Bazani, Matheus S. Barreto, Cristiane I. Cerceau, Daiane E. Blank, Maria J. M. Firmino, and Gustavo S. F. Souza.

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## Compliance with Ethical Standards

**Conflict of Interest** The authors declare that they have no conflict of interest.

**Ethical Approval** This article does not contain any studies with human and animal subjects.

**Informed Consent** Not applicable.

## References

- ABIC (2020) Associação Brasileira da Indústria de Café. <https://www.graogourmet.com/blog/24-de-maio-dia-nacional-cafe/>. Accessed 13 Jun 2019
- ABNT (2005) Brazilian Association of Technical Standards. NBR 14029: Agrochemicals - validation of analytical methods, Rio de Janeiro, p 19 (in Portuguese)
- ANVISA (2003) National Health Surveillance Agency, 2003. Resolution-RE no 899: guide for validation of analytical and bioanalytical methods, Brazil, p 15 (in Portuguese)
- Becker MM, Nunes GS, Ribeiro DB, FEPS S, Catanante G, Marty JL (2019) Determination of the antioxidant capacity of red fruits by miniaturized spectrometry assays. *J Braz Chem Soc* 30:1108–1114
- Benedetti LP d S, Dos Santos VB, Silva TA, Benedetti-Filho E, Martins VC, Fatibelo-Filho (2015) A digital image-based method employing a spot-test for quantification of ethanol in drinks. *Anal Methods* 7: 4138–4144. <https://doi.org/10.1039/c5ay00529a>
- Bravo L (1998) Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutr Rev* 56:317–333. <https://doi.org/10.1111/j.1753-4887.1998.tb01670.x>
- Cerceau CI, Barbosa LCA, Filomeno CA, Alvarenga ES, Demuner AJ, Fidencio PH (2016) An optimized and validated <sup>1</sup>H NMR method for the quantification of  $\alpha$ -pinene in essential oils. *Talanta* 150. <https://doi.org/10.1016/j.talanta.2015.10.087>
- Cerceau CI, Barbosa LCA, Alvarenga ES, Maltha CRA, Ismail FMD (2019) <sup>1</sup>H-NMR and GC for detection of adulteration in commercial essential oils of *Cymbopogon* ssp. *Phytochem Anal.* 31:88–97. <https://doi.org/10.1002/pca.2869>
- Cho MJ, Howard LR, Prior RL, Clark JR (2004) Flavonoid glycosides and antioxidant capacity of various blackberry, blueberry and red grape genotypes determined by high-performance liquid chromatography/mass spectrometry. *J Sci Food Agric* 84:1771–1782. <https://doi.org/10.1002/jsfa.1885>
- Choodum A, Kanatharana P, Wongniramaikul W, Daeid NN (2013) Using the iPhone as a device for a rapid quantitative analysis of trinitrotoluene in soil. *Talanta* 115:143–149. <https://doi.org/10.1016/j.talanta.2013.04.037>
- Clifford MN (2000) Chlorogenic acids and other cinnamates – nature, occurrence, dietary burden, absorption and metabolism. *J Sci Food Agric* 80:1033–1043. [https://doi.org/10.1002/\(sici\)1097-0010\(20000515\)80:7<1033::aid-jsfa595>3.3.co;2-k](https://doi.org/10.1002/(sici)1097-0010(20000515)80:7<1033::aid-jsfa595>3.3.co;2-k)
- Franco MOK, Suarez WT, Maia MV, Santos VB (2017) Smartphone application for methanol determination in sugar cane spirits employing digital image-based method. *Food Anal Methods* 10: 2102–2109. <https://doi.org/10.1007/s12161-016-0777-y>
- Helena FF, Queiroz MELR, Neves AA, Oliveira AF (2014) Otimização, validação e aplicação de método para determinação da concentração residual de difenoconazol em morangos após múltiplas aplicações. *Quim Nova* 37:153–157. <https://doi.org/10.1590/S0100-40422014000100025>
- Helfer GA, Magnus VS, Böck FC, Teichmann A, Ferrão MF, Costa AB (2017) PhotoMetrix: an application for univariate calibration and principal components analysis using colorimetry on mobile devices. *J Brazilian Chem Soc* 28:328–335
- Huang D, Boxin OU, Prior RL (2005) The chemistry behind antioxidant capacity assays. *J Agric Food Chem* 53:1841–1856. <https://doi.org/10.1021/jf030723c>
- IBGE (2017) Instituto Brasileiro de Geografia e Estatística. <https://agenciadenoticias.ibge.gov.br/agencia-sala-de-imprensa/2013-agencia-de-noticias/releases/19942-em-janeiro-ibge-preve-safra-6-0-inferior-a-de-2017>. Accessed 23 March 2019
- INMETRO (2011) National Institute of Metrology, Standardization and Industrial Quality, 2011. DOQ-CGCRE-008: guidelines on validations of methods of chemical, Brazil, p. 24 (in Portuguese)

- Intaravanne Y, Sumriddetchkajorn S, Nukeaw J (2012) Cell phone-based two-dimensional spectral analysis for banana ripeness estimation. *Sensors Actuators B Chem* 168:390–394. <https://doi.org/10.1016/j.snb.2012.04.042>
- Karadag A, Ozelcik B, Saner S (2009) Review of methods to determine antioxidant capacities. *Food Anal Methods* 2:41–60. <https://doi.org/10.1007/s12161-008-9067-7>
- Khoddami A, Wilkes MA, Roberts TH (2013) Techniques for analysis of plant phenolic compounds. *Molecules* 18:2328–2375. <https://doi.org/10.3390/molecules18022328>
- Lopez-molinero A, Liñan D, Sipiera D, Falcon R (2010) Chemometric interpretation of digital image colorimetry. Application for titanium determination in plastics. *Microchem J* 96:380–385. <https://doi.org/10.1016/j.microc.2010.06.013>
- Lyra WS, Santos VB, Dionízio AGG, Martins VL, Almeida LF, Nóbrega-Gaião E, Diniz PH, Silva EC, Araújo MC (2009) Digital image-based flame emission spectrometry. *Talanta* 77:1584–1589. <https://doi.org/10.1016/j.talanta.2008.09.057>
- Lyra W d S, Sanches FAC, Cunha FADS, Diniz PHGD, Lemos SG, Silva EC, Araujo MCU (2011) Indirect determination of sodium diclofenac, sodium dipyrone and calcium gluconate in injection drugs using digital image-based (webcam) flame emission spectrometric method. *Anal Methods* 3:1975–1980. <https://doi.org/10.1039/c1ay05197k>
- Melo EDA, Maciel MIS, Lima VLAG, Nascimento RJ (2008) Capacidade antioxidante de frutas. *Rev Bras Ciencias Farm* 44:193–201. <https://doi.org/10.1590/S1516-93322008000200005>
- Monošík R, Santos VB, Angnes L (2015) A simple paper-strip colorimetric method utilizing dehydrogenase enzymes for analysis of food components. *Anal Methods* 7:8177–8184. <https://doi.org/10.1039/c5ay01556a>
- Naczki M, Shahidi F (2006) Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. *J Pharm Biomed Anal* 41:1523–1542. <https://doi.org/10.1016/j.jpba.2006.04.002>
- Narita Y, Inouye K (2011) Inhibitory effects of chlorogenic acids from green coffee beans and cinnamate derivatives on the activity of porcine pancreas  $\alpha$ -amylase isozyme I. *Food Chem* 127:1532–1539. <https://doi.org/10.1016/j.foodchem.2011.02.013>
- Paciomik S, Yallouz AV, Campos RC, Gannerman D (2006) Scanner Image Analysis in the Quantification of Mercury using Spot-Tests. *J Brazilian Chem Soc* 17:156–161
- Paquet-durand O, Solle D, Schirmer M, Becker T, Hitzmann B (2012) Monitoring baking processes of bread rolls by digital image analysis. *J Food Eng* 111:425–431. <https://doi.org/10.1016/j.jfoodeng.2012.01.024>
- Ravazzi CG, Franco MOK, Vieira MCR, Suarez WT (2018) Smartphone application for captopril determination in dosage forms and synthetic urine employing digital imaging. *Talanta* 189:339–344. <https://doi.org/10.1016/j.talanta.2018.07.015>
- Santos PM, Pereira-Filho ER (2013) Digital image analysis – an alternative tool for monitoring milk authenticity. *Anal Methods* 5:3669–3674. <https://doi.org/10.1039/c3ay40561c>
- Santos MH, Lemos BB, Duarte SMS, Abreu CMP, Gouvêa CMCP (2007) Influência do processamento e da torrefação sobre a atividade antioxidante do café. *Quim Nova* 30:604–610
- Santos VB, Silva EKN, Oliveira LMA, Suarez WT (2019) Low cost in situ digital image method, based on spot testing and smartphone images, for determination of ascorbic acid in Brazilian Amazon native and exotic fruits. *Food Chem* 285:340–346. <https://doi.org/10.1016/j.foodchem.2019.01.167>
- Schwarz K, Bertelsen G, Nissen LR, Gardner PT, Heinonen MI, Hopia A, Huynh-Ba T, Lambelet P, McPhail D, Skibsted LH, Tijburg L (2001) Investigation of plant extracts for the protection of processed foods against lipid oxidation. Comparison of antioxidant assays based on radical scavenging, lipid oxidation and analysis of the principal antioxidant compounds. *Eur Food Res Technol* 212:319–328. <https://doi.org/10.1007/s002170000256>
- Sena RC, Soares M, Pereira MLO, Silva RCD, Rosário FF, Silva JFC (2011) A simple method based on the application of a CCD camera as a sensor to detect low concentrations of barium sulfate in suspension. *Sensors* 11:864–875. <https://doi.org/10.3390/s110100864>
- Shadkani F, Estevez S, Helleur R (2009) Analysis of catechins and condensed tannins by thermally assisted hydrolysis/methylation-GC/MS and by a novel two step methylation. *J Anal Appl Pyrolysis* 85:54–65. <https://doi.org/10.1016/j.jaap.2008.09.001>
- Sorouraddin M-H, Saadati M, Mirabi F (2015) Simultaneous determination of some common food dyes in commercial products by digital image analysis. *J Food Drug Anal* 23:447–452. <https://doi.org/10.1016/j.jfda.2014.10.007>
- Stalikas CD (2007) Extraction, separation, and detection methods for phenolic acids and flavonoids. *J Sep Sci* 30:3268–3295. <https://doi.org/10.1002/jssc.200700261>
- Stalmach A, Mullen W, Nagai C, Crozier A (2006) On-line HPLC analysis of the antioxidant activity of phenolic compounds in brewed, paper-filtered coffee. *Brazilian J Plant Physiol* 18:253–262. <https://doi.org/10.1590/S1677-04202006000100018>
- Trugo LC, Macrae R (1984) Chlorogenic acid composition of instant coffees. *Analyst* 109:263–266
- Yen G-C, Chen H-Y (1995) Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J Agric Food Chem* 43:27–32. <https://doi.org/10.1021/jf00049a007>

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