$\left($ ce) BY

Using smartphone for monitoring colorimetric reactions aiming at determining antioxidant activity

Daniela de Quadros PEDROSO¹ ^{(D}[,](https://orcid.org/0000-0002-0743-8641) Luana Estefani KNAUL¹ ^{(D}, Marcelo Nepomoceno KAPP¹ (D, Caroline da Costa Silva GONÇALVES¹ (D[,](https://orcid.org/0000-0001-9154-7930)Marcela BOROSKI^{1*}

Abstract

The use of technology for antioxidant determination in foods has contributed toward enhancing the applicability of this analysis in *in vitro* assays. The present study sought to evaluate the activity of antioxidants in food products using the following analytical methodologies: analysis of total phenols, flavonoids, ABTS, DPPH, reducing power by potassium ferricyanide, and FRAP, using the smartphone app PhotoMetrix PRO®. These methodologies underwent analytical validation demonstrating linearity at 95% confidence interval, while residues displayed homoscedasticity and random distribution. The values obtained for the limit of detection (LOD) and limit of quantification (LOQ) were below the working range for all methodologies. The correlation coefficients obtained for the curves were above 0.99, except for the FRAP method. For the analysis, the values obtained for relative standard deviation (RSD %) for repeatability and intermediate precision were lower than 5%, except for ABTS and DPPH analysis, which presented values lower than 10%. The PhotoMetrix PRO® app has proven to be efficient for use in the analysis of whole grape juice samples, when compared to UV-VIS spectrophotometer.

Keywords: digital image analysis; grape juice; mobile app; antioxidants.

Practical Application: The smartphone app was found to be highly efficient for monitoring antioxidant analysis.

1 INTRODUCTION

Antioxidants are bioactive compounds that act in biological systems and in foods by slowing down or preventing the oxidation of biomolecules such as lipids or proteins. Assessing the potential of antioxidants in biological systems often involves *in vitro* assays using macromolecules that may or may not resemble physiological systems; these assays are used to correlate the expected effects with antioxidant ingestion through *in vitro* laboratory analyses (Siddeeg et al., 2021).

In view of that, colorimetric analyses have gained prominence for monitoring different classes of compounds and analyzing their antioxidant capacity. Some of the colorimetric methods applied for the analysis of antioxidant compounds include the following: analysis of total phenolic compounds; analysis of flavonoids; 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS); assays for the capture of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical; analysis of the reducing power by potassium ferricyanide; and the analysis of Ferric Reducing Ability Power (FRAP) (Boroski et al., 2015; Caramês et al., 2020; Stratil et al., 2006). The key advantages of the aforementioned analytical techniques include their animal-free nature, relatively low cost, simplicity of execution without requiring highly trained analysts, and ease of equipment operation (Anh-Dao et al., 2023; Stratil et al., 2006; Thongsuk & Sameenoi, 2022).

In recent years, the application of colorimetric techniques for the analysis of antioxidant compounds has evolved alongside the advancement of digital imaging technologies, including smartphones, digital cameras, webcams and scanners, as well as up-to-date software for the treatment and processing of images (Bazani et al., 2021; Ledesma et al., 2019; Minh-Huy et al., 2023), where colorimetric analysis is conducted based on digital imaging (Bazani et al., 2021; Ledesma et al., 2019; Minh-Huy et al., 2023). This alternative approach, which involves the use of digital resources, simplified the assessment of various samples, making it faster, more practical, cost-effective, accessible, and efficient to perform (Al-Nidawi & Alshana, 2021; Caleb et al., 2021; dos Santos et al., 2019; Minh-Huy et al., 2023).

The digital imaging-based colorimetric technique has been increasingly applied for the analysis of a wide range of analytes, including the determination of total phenolic content and antioxidants in tea (Minh-Huy et al., 2023), grape juice (Caramês et al., 2017), beer (Ledesma et al., 2019), coffee (Anh-Dao et al., 2023; Bazani et al., 2021), strawberries (Bazani et al., 2021), tomatoes (Bazani et al., 2021), ascorbic acid in fruits native to the Brazilian Amazon such as *bacuri*, *cupuaçu*, *muruci*, *cajá*, cashew, mango, orange, and passion fruit (dos Santos et al., 2019), polyphenols in wines (Vallejos et al., 2019), alkaline phosphatase in raw milk (Mahato & Chandra, 2019), curcumin in turmeric and tea samples

Received Nov 20, 2023.

*Corresponding author: marcela.boroski@unila.edu.br

Accepted Dec 11, 2023.

¹ *Universidade Federal da Integração Latino-Americana, Foz do Iguaçu, PR, Brazil.*

Funding: PRPPG/UNILA, CNPq, and CAPES, provided under the research funding call announcement No. 77/2022 PRPPG.

(Caleb & Alshana, 2021), and iodate in table salt samples (Caleb et al., 2021). In addition, the digital imaging-based colorimetric technique has also been applied for the determination of mercury ions in drinking water (Firdaus et al., 2019) and iron ions in pharmaceutical formulations (Santos et al., 2021).

The main objective of the present study was to analytically validate the *in vitro* methodologies employed for the analysis of antioxidant activity of foods using a smartphone app to monitor the colorimetric reactions and to process the results obtained from this analysis.

2 MATERIAL AND METHODS

2.1 Reagents and materials

The reagents employed for the conduct of the experiments included the following: gallic acid (99%, Neon), sodium carbonate (99%, *Êxodo Científica*), methanol PA (Sal-R), Folin-Ciocalteu phenolic reagent (2 N, IMBRALAB), aluminum chloride (99%, *Dinâmica*), acetone PA (Synth), quercetin (99%, Sigma-Aldrich), DPPH $(C_{18}H_{12}N_5O_6, 2,2$ -diphenyl-1-picrylhydrazyl, 99.9% Sigma-Aldrich), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), diamonic salt (99%, Sigma-Aldrich), ethanol PA (Sal-R), potassium persulfate (99%, *Dinâmica*), Trolox reagent (99%, Sigma-Aldrich), monobasic potassium phosphate (Neon), bibasic potassium phosphate (*Êxodo Científica*), hydrochloric acid 37% PA (CRQ), trichloroacetic acid (99.2%, Neon), potassium ferricyanide (99%, *Sinergia Científica*), ferric chloride (99.5%, *Dinâmica*), sodium acetate buffer solution (300 mmol L¹, pH 3.6), ferrous sulfate (99%, *Dinâmica*), 2,4,6-tripyridyl-1,3,5-triazine (TPTZ) (99%, Sigma-Aldrich). Distilled water was used to prepare the solutions (Type II, Purelab Option Q system). A sample of whole grape juice obtained from the local market was used for the analysis of the methodologies (Foz do Iguazu, Brazil).

The experiments were conducted using the following set of equipment: pH meter (Ohaus, Starter 3100M), UV-Vis spectrophotometer (Thermo Fisher Scientific, Evolution 201), and cylindrical glass tubes of 10 cm with internal diameter of 1.2 mm and glass cubettes.

2.2 Monitoring the colorimetric reactions

The *in vitro* methods for the analysis of antioxidant activity were executed using UV-VIS spectrophotometer and the *smartphone* app PhotoMetrix PRO®.

The images were captured using the Samsung Galaxy S21 5G cell phone (model SM-G991B with 64-megapixel camera) equipped with the PhotoMetrix PRO®app. To capture the images through the smartphone app, the following procedures were executed: Univariate Analysis, Vetor RGB, Calibration (to plot the analytical curve) or Sampling (for the analysis of the samples on the curve). The analyses of the samples via the PhotoMetrix PRO® app were carried out using 10 cm cylindrical glass tubes with internal diameter of 1.2 mm.

The analyses were conducted using the apparatus shown in Figure 1, which was used for light control and the proper

Figure 1. Apparatus used for constructing the analytical curve and for the analysis of the samples through the PhotoMetrix PRO® app.

positioning of the samples. The apparatus consisted of a wooden box with the interior coated in black ink, sheets of sulfite paper to ensure the contrast of the colorimetric reactions, LED cables connected to a 9V battery cell used as a power source, and a support for securing the smartphone onto the surface of the box (de Lourenço et al., 2021).

2.3 Validation of the methodologies

During the study, the colorimetric methods were subjected to the following modifications: the volumes of the originally proposed methods were reduced; the monitoring of the colorimetric reactions was performed using the smartphone app PhotoMetrix PRO®.

Linearity was evaluated through the construction of six (6) analytical curves prepared in triplicate. The homoscedasticity of the residues was evaluated at 95% significance level. Limits of detection (LOD) and quantification (LOQ) were determined through the reading of 7 replicates of the analytical blank, considering $3.3 \times \sigma/m$ and $10 \times \sigma/m$, respectively. Where σ is the standard deviation of ten blank measurements and m is the slope of the analytical curve (AOAC, 2016; INMETRO, 2016).

For the precision analysis, which is determined through the study of repeatability or intermediate precision, three concentration levels were used. Precision was estimated based on relative standard deviation (RSD %). Intermediate precision was estimated based on the adoption of an interval of 72 h between the analyses.

The methodologies developed in the present study, which involved using the PhotoMetrix® smartphone app for monitoring antioxidant activity, were compared with the conventional method based on the application of UV-VIS spectrophotometer. The mean values obtained were compared using the Student's *t-*test for paired data at a 95% confidence level. The graphics obtained for the results of the analyses were plotted using Excel® .

2.4 Total phenolic compounds

The analysis of total phenolic compounds was carried out based on the detection of reduced substances using the Folin-Ciocalteu reagent (FCR) (Singleton & Rossi, 1965). A solution containing 200 mg L-1 of gallic acid was prepared in distilled water, and dilutions were carried out for the construction of analytical curve in concentrations between 25 and 150 mg L-1. An aliquot of 200 μL of each standard solution was transferred to a test tube, and the following substances were added into the solution: 250 μL of diluted FCR (1:1 v/v in distilled water), 500 μL of saturated Na₂CO₃ solution and 4 mL of distilled water. The test tubes were agitated and kept at room temperature under light protection for 25 min and were subsequently centrifuged for 10 min at 3,000 rpm. For the blank solution employed in the analysis, the volume of the standard solution was replaced by distilled water. Additionally, 250 μL of 10% whole grape juice (v/v in distilled water) was used for the analysis of real sample. Absorbance was determined using a UV-VIS spectrophotometer at 725 nm.

2.5 Total flavonoids

Total flavonoids were determined through the analysis of complexation reaction involving the antioxidants and aluminum metal, based on the formation of a yellow color complex. This analysis was performed based on the method proposed by Dowd (1959), with some adaptations. The analytical curve was prepared by diluting 10 mg of quercetin in a mixture containing 2 mL of methanol and 3 mL of acetone (for complete solubilization), resulting in a final concentration of 2,000 mg L-1. Dilutions were made from this solution and six points of the curve were prepared in concentrations ranging from 10 to 100 mg L-1. An amount of 500 μL of each standard solution was transferred to test tubes; followed by the addition of 250 μL of 5% AICI_{3} (m/v in methanol) and 4.25 mL of methanol. The tubes were homogenized and kept at room temperature under light protection for 30 min. A blank solution was also prepared by replacing the aliquot of the standard solution by methanol, and the same solution volume was used for the sample of 10% whole grape juice (v/v in distilled water). Absorbance was measured using UV-VIS spectrophotometer at 425 nm.

2.6 DPPH

The assay related to the capture of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was carried out based on the analysis of the oxidation reaction of the DPPH radical with the analyzed sample. The antioxidant potential of the sample is found to be proportional to the discoloration of the DPPH solution — which exhibits a range of color change from purple to yellow. The analytical methodology employed in the study was developed based on the original methodologies proposed by Brand-Williams et al. (1995) and Kirigaya et al. (1971). A solution of 200 mg L-1 of gallic acid was prepared in distilled water, and dilutions were made for the construction of the analytical curve in the concentrations ranging from 35 mg L⁻¹ to 150 mg L⁻¹. 4,000 µL solution of 47 mg L-1 DPPH (prepared with methanol) and 100 μL of each standard solution were placed in test tubes. The tubes were homogenized and kept at room temperature for 30 min. For the blank solution used for the analysis, the volume of the standard solution was replaced by methanol, and the same procedure was applied for the sample of 10% whole grape juice (v/v in distilled water). All analyses were performed under light protection. Absorbance was measured using UV-VIS spectrophotometer at 517 nm.

The results were obtained based on the estimate of IC_{50} , which determines the concentration of antioxidant required to inhibit 50% of DPPH radical. To determine the IC_{50} values, the percentage of DPPH• relative to the standard solution or sample was calculated as illustrated in Equation 1 below.

$$
\% DPPH^{\bullet} Inhibition = \frac{(AbsDPPH^{\bullet} - Abssample)}{AbsDPPH^{\bullet}} 100 \qquad (1)
$$

Where:

AbsDPPH: Absorbance of the methanol solution in the DPPH radical;

Abssample: Absorbance of the sample after 30 min of reaction with the DPPH[•] solution.

2.7 ABTS

The analysis of the antioxidant activity of the sample was carried out using the technique involving the application of the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical, based on the method proposed by Rice-Evans and Miller (1994), with some adaptations. The analytical curve was prepared using 2,000 μmol L⁻¹ Trolox 6-hydroxy-2,5,7,8-tetramethylcromano-2-carboxylic acid) standard solution (prepared in ethanol). This solution was subjected to dilutions, where concentrations ranging from 380 to 1,500 μmol L-1 were obtained. An aliquot of 2,000 μL of the ABTS•+ radical cation solution was transferred to test tubes and 20 μL of the Trolox standard solutions were added to the tubes. The ABTS^{*+} radical cation was prepared by diluting ABTS in a 2.45 mmol L-1 potassium persulfate solution, which was kept idle for 16 h under light protection. Subsequently, it was diluted with potassium phosphate buffer solution (pH 7.40) until an absorbance ranging from 0.680 to 0.720 at 734 nm was obtained (using UV-VIS spectrophotometer). The test tubes were homogenized and kept at room temperature under light protection for 6 min. A blank solution was also prepared by replacing the aliquot of the standard solution with phosphate buffer solution; the same procedure was executed for the 10% whole grape juice sample (v/v in distilled water). Absorbance was measured using a UV-VIS spectrophotometer at 734 nm.

2.8 Reducing power by potassium ferricyanide

The analysis of reducing power by potassium ferricyanide assesses the ability to reduce Fe^{3+} (iron III) ion to Fe^{2+} (iron II). This analysis was performed using the methodology developed by Berker et al. (2007). The analytical curve was constructed using the standard solution of gallic acid at 200 mg $L⁻¹$ in concentrations ranging from 10 to 100 mg L^{-1} . An amount of 1,000 μ L of the standard solution was placed in test tubes, followed by the addition of 2.50 mL of phosphate buffer solution at pH 7.0 and 2.50 mL of 1% potassium ferricyanide solution (m/v in distilled water). The test tubes were then placed in a heating bath at 50 °C for 20 min, and 2.50 mL of 10% trichloroacetic acid solution (m/v in distilled water) was added to the tubes. After that, the tubes were centrifuged at 3,000 rpm for 10 min, and 2.50 mL of the supernatant was removed and transferred to other tubes, where

2.50 mL of distilled water and 0.50 mL of 0.10% ferric chloride solution (w/v in distilled water) were added therein. A blank solution was also prepared by replacing the aliquot of the standard solution with distilled water. The same solution volume of this solution was applied for the sample of 10% whole grape juice (m/v in distilled water). Absorbance was immediately measured using a UV-VIS spectophotometer at 700 nm.

2.9 Ferric reducing ability power

The FRAP test indirectly measures the total antioxidant activity of a sample through the reduction of the complex formed by TPTZ, which presents an intense blue color. This analysis followed the methodology proposed by Moon and Shibamoto (2009), with some adaptations. The analytical curve was constructed using the standard solution of 2,000 μmol L-1 ferrous sulfate (heptahydrate), which was diluted, obtaining concentration levels ranging between 750 and 1,750 μ mol L⁻¹. To conduct the analysis, an amount of 3,000 μ L [Fe³⁺(TPTZ)₂]Cl₃ (FRAP reagent) solution which was prepared by mixing 100 mL acetate buffer solution at 300 mmol $L^1(pH 3.60)$, 10 mL TPTZ solution at 10 mmol L^1 (in distilled water), and 10 mL ferric chloride solution (hexahydrate) at 20 mmol L^{-1} — was poured into test tubes, and this was followed by the addition of 100 μL of the standard solution and 300 μL of distilled water therein. The tubes were then placed in a heating bath at 37 °C for 5 min. A blank solution was also prepared by replacing the aliquot of the standard solution with distilled water, and the same solution volume was applied for the sample of 10% whole grape juice (v/v in distilled water). Absorbance was measured using UV-VIS spectrophotometer at 593 nm.

3 RESULTS AND DISCUSSION

3.1 Analytical validation

The methodologies for the analysis of antioxidant capacity in foods have been well consolidated in the literature. The application of advanced technology in routine lab analyses has helped accelerate these analyses and has reduced both the time and the complexity of the procedure. In addition, the miniaturization of analyses has played a significant role in lowering costs and minimizing waste production in the laboratories. To ensure the integrity of the results, it is essentially important that the methods be subjected to validation after modification and optimization procedures (AOAC, 2016; INMETRO, 2016).

Linearity assessment of the methods was conducted through analysis of variance using the spreadsheet developed by Ribeiro et al. (2008). The analytical curves exhibited linearity at 95% confidence interval, while the residues displayed homoscedasticity and a random distribution. The correlation coefficients obtained for the curves were above 0.99, except for the FRAP method (Table 1). For the analysis of total phenolic compounds, flavonoids, ABTS, reducing power by potassium ferricyanide, and reducing power by the FRAP method, the linearity was obtained by plotting the concentration of the standard solutions as a function of the signal obtained from the smartphone. For the analysis involving the DPPH[·] radical assays, the signal values were replaced with the percentage of inhibition of the radical.

In order to compare the applicability of the methods in food samples, LOD and LOQ were estimated. The values obtained fell below the working range for all the methodologies; this result pointed to the feasibility of the methods in terms of application for the determination of antioxidant activity of analytes at low concentrations. When it comes to the quantitative determination of the analysis of antioxidants, the limits of detection of the methods are particularly important. Samples with high antioxidant activity are found to be easily diluted; it should be noted however that the preparation of samples is a limiting factor when bioactive compounds are present in low concentrations in the composition.

Other studies reported in the literature have demonstrated the efficacy of digital apps in detecting and quantifying analytical curves with low concentrations, particularly in analyzing the antioxidant capacity of extracts of tomatoes, strawberries, and coffee (Bazani et al., 2021), tea and herbal infusions (Calabria et al., 2021), and the detection of nitrite and ammonia in river, waste and sea water samples (Zheng et al., 2022). It is worth pointing out that the PhotoMetrix PRO® smartphone app has been previously validated for the analysis of other substances in a wide range of samples; determination of iron in vitamins, where the authors obtained LOD and LOQ of 0.19 mg L⁻¹ and 0.23 mg L⁻¹, respectively (Helfer et al., 2016); determination of fluoride in water, where the authors obtained LOD and LOQ of 0.05 mg L^{-1} and 0.10 mg L^{-1} , respectively; and for the determination of phosphorus in water, yelding LOD and LOQ of 0.010 mg $L⁻¹$ and 0.019 mg $L⁻¹$, respectively (Pappis et al., 2019); speciation of chromium and determination of Cr (VI) in leather samples, where the authors obtained LOD and LOQ of 0.6 mg $L⁻¹$ and 0.2 mg kg⁻¹, respectively (Costa et al., 2019); and for the determination of the content of total phenols and antioxidants in tomatoes, strawberries, and coffee, with LOD and LOQ of 0.03 mg L^{-1} and 0.11 mg L^{-1} for total phenolic compounds and 0.26 mg L^{-1} and 0.88 mg L-1 for antioxidants based on the test of reducing power for the reduction of the 2,6-di- tert-butyl-4-methylphenol (BHT) standard (Bazani et al., 2021). Essentially, the LOD and LOQ values from these studies show that the PhotoMetrix PRO® app is capable of detecting and quantifying analytes present in a wide range of samples, even at trace concentrations.

 The precision of the methodologies was evaluated through repeatability and intermediate precision analyses across three concentration levels for each analysis (Table 1). It should be noted that the use of the apparatus for capturing images was essentially important for obtaining better results, as it helped prevent interferences, such as ambient light and unwanted movements during image capture, while ensuring a consistent distance between the camera and the analyzed sample.

The results obtained in this study are in line with the regulatory guidelines that recommend that the relative standard deviation percentage (% RSD) for concentrations of 100, 10, 1, and 0.1 mg L-1 should be equal or inferior to 5.3, 7.3, 11.0, and 15.0, respectively (AOAC, 2016; INMETRO, 2016).

3.2 Real sample analysis

Table 2 shows the results obtained from the analysis conducted using the samples of whole grape juice.

Table 1. Results obtained from the analyses conducted using the proposed smartphone app*.*

Analysis	Range	\mathbb{R}^2	LOD	LOO	Repeatability RSD(%)	Intermediate precision $RSD(\%)$
Total Phenols (mg L^{-1})	$10 - 150$	0.998	1.41	4.26	3.81	4.50
Flavonoids (mg L^{-1})	$20 - 100$	0.992	0.76	2.32	0.68	4.18
$DPPH (mg L-1)$	$59 - 155$	0.995	13.01	39.44	8.14	3.51
ABTS (μ mol L ⁻¹)	$380 - 1,500$	0.998	57.92	175.51	7.01	6.44
$\mathrm{RPPF}^\mathrm{d}\left(\mathrm{mg}\ L^\text{-1}\right)$	$20 - 100$	0.993	0.52	1.57	2.77	1.77
FRAP (μ mol L ⁻¹)	$750 - 1,750$	0.970	210.31	637.29	2.23	2.31

LOD: limit of detection; LOQ: limit of quantification; RSD: relative standard derivation *n* = 9; DPPH:2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); RPPF: reducing power by potassium ferricyanide; FRAP: ferric reducing ability power.

Table 2. Data obtained for the extract concentrations relative to the methodologies applied.

Analysis	Spectrophotometer UV-Vis	PhotoMetrix PRO [®]	T-Test
Total Phenols (mg GAE L ⁻¹)	$1,429.10 \pm 89.63$	$1,392.49 \pm 58.50$	2.66 ^a
Flavonoids (mg $QE L^{-1}$)	99.34 ± 1.56	132.73 ± 3.91	22.85 ^b
DPPH $(IC_{50}$ mg $L^{-1})$	2.82 ± 0.00	4.55 ± 0.00	76.29 ^b
ABTS (mmol TE L^{-1})	22.87 ± 0.78	28.99 ± 0.98	7.49 ^b
R PPF (mg GAE L^{-1})	893.9 ± 9.12	907.56 ± 4.56	1.81 ^a
$FRAP$ (mmol $SFe^{+2}E L^{-1}$)	14.24 ± 0.26	16.63 ± 0.11	18.06 ^b

The results are expressed as mean ± standard deviations (*n* = 3); GAE: gallic acid equivalent; QE: quercentin equivalent; DPPH: 2,2-diphenyl-1-picrylhydrazyl; IC₅₀: antioxidant concentration required to inhibit 50% of DPPH• radical; ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); TE: Trolox equivalent; RPPF:reducing power by potassium ferricyanide; FRAP: ferric reducing ability power; SFe*2E: FeSO₄ equivalent; *Similar methodology; ^bdifferent methodologies.

Based on the concentrations of the samples recorded for the methodologies related to the analysis of total phenolic compounds, flavonoids, ABTS, DPPH, reducing power by ferricyanide, and reducing power by FRAP under the application of the UV-VIS spectrophotometer and the PhotoMetrix PRO® smartphone app, Student's *t-*test results were obtained (Table 2). For the results that are statistically similar at 95% confidence level with 2 degrees of freedom, the methodologies required a *t*-value lower than the *t*-critical value (4.30). Based on the Student's *t-*test results, only the methodologies involving the analysis of total phenolic compounds and reducing power by potassium ferricyanide exhibited statistical similarity between the data obtained from the application of the UV-VIS spectrophotometer and the PhotoMetrix PRO® app, as observed in Table 2. The differences observed between the other methodologies can be attributed to interferences from external factors in the smartphone app's apparatus, including battery capacity and changes of position of the electronic gadget during replicates.

4 CONCLUSION

The methodologies employed in this study for the analysis of antioxidant activity based on the application of the PhotoMetrix PRO® smartphone app for monitoring colorimetric reactions yielded satisfactory results in comparison with the UV-VIS spectrophotometric method. Regarding linearity, there was a clear similarity in terms of the behavior of the analytical curves constructed using the PhotoMetrix PRO® app and the UV-VIS spectrophotometer. All the analyses conducted in this study fell within the parameters assessed in the validation analysis for the two techniques investigated.

The PhotoMetrix PRO® app has proven to be efficient for use in the analysis of whole grape juice samples under the application of the methodologies involving the analysis of total phenolic compounds, flavonoids, DPPH, ABTS, and reducing power by potassium ferricyanide and by the FRAP method. Considering that the analytical device proposed in this study is of low cost and easily accessible, it can be incorporated into the techniques applied for the analysis of antioxidant activity in teaching and research laboratories.

ACKNOWLEDGMENTS

The authors are grateful to the LEIMAA research group.

REFERENCES

- Al-Nidawi, M., & Alshana, U. (2021). Reversed-phase switchable-hydrophilicity solvent liquid-liquid microextraction of copper prior to its determination by smartphone digital image colorimetry. *Journal of Food Composition and Analysis*, *104*, 104140. [https://](https://doi.org/10.1016/j.jfca.2021.104140) doi.org/10.1016/j.jfca.2021.104140
- Anh-Dao, L. T., Thanh-Nho, N., Huu-Trung, B., Tien-Giang, N., Ut Dong, T., Quoc-Duy, N., Quang-Hieu, N., Le-Vy, N., Thanh-Dieu, N. T., To, D. V. T., Minh-Huy, D., & Cong-Hau, N. (2023). A portable colorimetric tool using a smartphone camera applied for determining total phenolic contents in coffee products. *Chinese Journal of Analytical Chemistry*, *51*(3), 100228. [https://doi.](https://doi.org/10.1016/j.cjac.2023.100228) [org/10.1016/j.cjac.2023.100228](https://doi.org/10.1016/j.cjac.2023.100228)
- Association of Official Analytical Chemists (AOAC) (2016). *Official methods of analysis of the Association of Official Analytical Chemists*. AOAC. Retrieved from [https://www.techstreet.com/](https://www.techstreet.com/standards/official-methods-of-analysis-of-aoac-international-20th-edition-2016?product_id=1937367#full) [standards/official-methods-of-analysis-of-aoac-internation](https://www.techstreet.com/standards/official-methods-of-analysis-of-aoac-international-20th-edition-2016?product_id=1937367#full)[al-20th-edition-2016?product_id=1937367#full](https://www.techstreet.com/standards/official-methods-of-analysis-of-aoac-international-20th-edition-2016?product_id=1937367#full)
- Bazani, E. J. O., Barreto, M. S., Demuner, A. J., Santos, M. H., Cerceau, C. I., Blank, D. E., Firmino, M. J. M., Souza, G. S. F., Franco, M. O. K., Suarez, W. T., & Stringheta, P. C. (2021). Smartphone Application for Total Phenols Content and Antioxidant Determination in Tomato, Strawberry, and Coffee Employing Digital Imaging. *Food Analytical Methods*, *14*(4), 631-640. [https://doi.org/10.1007/](https://doi.org/10.1007/s12161-020-01907-z) [s12161-020-01907-z](https://doi.org/10.1007/s12161-020-01907-z)
- Berker, K. I., Güçlü, K., Tor, İ., & Apak, R. (2007). Comparative evaluation of Fe(III) reducing power-based antioxidant capacity assays in the presence of phenanthroline, batho-phenanthroline, tripyridyltriazine (FRAP), and ferricyanide reagents. *Talanta*, *72*(3), 1157-1165.<https://doi.org/10.1016/j.talanta.2007.01.019>
- Boroski, M., Visentainer, J. V., Cottica, S. M., & Morais, D. R. (2015). *Antioxidantes: Princípios e Métodos Analíticos*. Appris.
- Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, *28*(1), 25-30. [https://doi.org/10.1016/](https://doi.org/10.1016/S0023-6438(95)80008-5) [S0023-6438\(95\)80008-5](https://doi.org/10.1016/S0023-6438(95)80008-5)
- Calabria, D., Guardigli, M., Severi, P., Trozzi, I., Pace, A., Cinti, S., Zangheri, M., & Mirasoli, M. (2021). A Smartphone-Based Chemosensor to Evaluate Antioxidants in Agri-Food Matrices by In Situ AuNP Formation. *Sensors*, *21*(16), 5432. [https://doi.](https://doi.org/10.3390/s21165432) [org/10.3390/s21165432](https://doi.org/10.3390/s21165432)
- Caleb, J., & Alshana, U. (2021). Supramolecular solvent-liquid-liquid microextraction followed by smartphone digital image colorimetry for the determination of curcumin in food samples. *Sustainable Chemistry and Pharmacy*, *21*, 100424. [https://doi.org/10.1016/j.](https://doi.org/10.1016/j.scp.2021.100424) [scp.2021.100424](https://doi.org/10.1016/j.scp.2021.100424)
- Caleb, J., Alshana, U., & Ertaş, N. (2021). Smartphone digital image colorimetry combined with solidification of floating organic drop-dispersive liquid-liquid microextraction for the determination of iodate in table salt. *Food Chemistry*, *336*, 127708. [https://](https://doi.org/10.1016/j.foodchem.2020.127708) doi.org/10.1016/j.foodchem.2020.127708
- Caramês, E. T. S., Alamar, P. D., & Lima Pallone, J. A. (2020). Bioactive Compounds and Antioxidant Capacity in Freeze-Dried Red Cabbage by FT-NIR and MIR Spectroscopy and Chemometric Tools. *Food Analytical Methods*, *13*(1), 78-85. [https://doi.org/10.1007/](https://doi.org/10.1007/s12161-019-01523-6) [s12161-019-01523-6](https://doi.org/10.1007/s12161-019-01523-6)
- Caramês, E. T. S., Alamar, P. D., Poppi, R. J., & Pallone, J. A. L. (2017). Rapid Assessment of Total Phenolic and Anthocyanin Contents in Grape Juice Using Infrared Spectroscopy and Multivariate Calibration. *Food Analytical Methods*, *10*(5), 1609-1615. [https://](https://doi.org/10.1007/s12161-016-0721-1) doi.org/10.1007/s12161-016-0721-1
- Costa, V., Neiva, A., & Pereira-Filho, E. (2019). Chromium speciation in leather samples: An experiment using digital images, mobile phones and environmental concepts. *Eclética Química Journal*, *44*(1), 62-74. [https://doi.org/10.26850/1678-4618eqj.v44.1.2019.](https://doi.org/10.26850/1678-4618eqj.v44.1.2019.p62-74) [p62-74](https://doi.org/10.26850/1678-4618eqj.v44.1.2019.p62-74)
- De Lourenço, E. C., De Paula, S., De Setti, G. O., Toci, A. T., Padilha, J. C., Da Silva, E. M., & Boroski, M. (2021). Determination of iron content using the PhotoMetrix PRO® application: Technology in favor of teaching chemistry. *Revista Virtual de Química*, *13*(1), 192-206. <https://doi.org/10.21577/1984-6835.20200137>
- dos Santos, V. B., da Silva, E. K. N., de Oliveira, L. M. A., & Suarez, W. T. (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 Chemistry*, *285*, 340-346. <https://doi.org/10.1016/j.foodchem.2019.01.167>
- Dowd, L. E. (1959). Spectrophotometric Determination of Quercetin. *Analytical Chemistry*, *31*(7), 1184-1187. [https://doi.org/10.1021/](https://doi.org/10.1021/ac60151a033) [ac60151a033](https://doi.org/10.1021/ac60151a033)
- Firdaus, M. L., Aprian, A., Meileza, N., Hitsmi, M., Elvia, R., Rahmidar, L., & Khaydarov, R. (2019). Smartphone Coupled with a Paper-Based Colorimetric Device for Sensitive and Portable Mercury Ion Sensing. *Chemosensors*, *7*(2), 25. [https://doi.org/10.3390/](https://doi.org/10.3390/chemosensors7020025) [chemosensors7020025](https://doi.org/10.3390/chemosensors7020025)
- Helfer, G. A., Magnus, V. S., Böck, F. C., Teichmann, A., Ferrão, M. F., & Costa, A. B. da (2016). PhotoMetrix: An Application for Univariate Calibration and Principal Components Analysis Using Colorimetry on Mobile Devices. *Journal of the Brazilian Chemical Society*, *28*(2), 328-335. <https://doi.org/10.5935/0103-5053.20160182>
- Instituto Nacional de Metrologia, Qualidade e Tecnologia (INMETRO) (2016). Orientação Sobre Validação de Métodos Analíticos, Brazil. INMETRO. Retrieved from [http://www.inmetro.gov.br/Sidoq/](http://www.inmetro.gov.br/Sidoq/Arquivos/Cgcre/DOQ/DOQ-Cgcre-8_05.pdf) [Arquivos/Cgcre/DOQ/DOQ-Cgcre-8_05.pdf.](http://www.inmetro.gov.br/Sidoq/Arquivos/Cgcre/DOQ/DOQ-Cgcre-8_05.pdf)
- Kirigaya, N., Kato, H., & Fujimaki, M. (1971). Studies on antioxidant activity of nonenzymic browning reaction products III. *Nippon Nogeikagaku Kaishi*, *45*(6), 292-298. [https://doi.org/10.1271/](https://doi.org/10.1271/nogeikagaku1924.45.292) [nogeikagaku1924.45.292](https://doi.org/10.1271/nogeikagaku1924.45.292)
- Ledesma, C. M., Krepsky, L. M., & Borges, E. M. (2019). Using a Flatbed Scanner and Automated Digital Image Analysis To Determine the Total Phenolic Content in Beer. *Journal of Chemical Education*, *96*(10), 2315-2321.<https://doi.org/10.1021/acs.jchemed.8b01037>
- Mahato, K., & Chandra, P. (2019). Paper-based miniaturized immunosensor for naked eye ALP detection based on digital image colorimetry integrated with smartphone. *Biosensors and Bioelectronics*, *128*, 9-16.<https://doi.org/10.1016/j.bios.2018.12.006>
- Minh-Huy, D., Anh-Dao, L.-T., Thanh-Nho, N., Nhon-Duc, L., & Cong-Hau, N. (2023). Smartphone-based digital images as a lowcost and simple colorimetric approach for the assessment of total phenolic contents in several specific Vietnamese dried tea products and their liquors. *Food Chemistry*, *401*, 134147. [https://doi.](https://doi.org/10.1016/j.foodchem.2022.134147) [org/10.1016/j.foodchem.2022.134147](https://doi.org/10.1016/j.foodchem.2022.134147)
- Moon, J.-K., & Shibamoto, T. (2009). Antioxidant Assays for Plant and Food Components. *Journal of Agricultural and Food Chemistry*, *57*(5), 1655-1666.<https://doi.org/10.1021/jf803537k>
- Pappis, C., Librelotto, M., Baumann, L., Parckert, A. B., Santos, R. O., Teixeira, I. D., Helfer, G. A., Lobo, E. A., & da Costa, A. Ben. (2019). Point-of-use determination of fluoride and phosphorus in water through a smartphone using the PhotoMetrix® App. *Brazilian Journal of Analytical Chemistry*, *6*(25), 58-66. [https://](https://doi.org/10.30744/BRJAC.2179-3425.TN-25-2019) doi.org/10.30744/BRJAC.2179-3425.TN-25-2019
- Rice-Evans, C., & Miller, N. J. (1994). Total antioxidant status in plasma and body fluids. *Methods in Enzymology*, *234*, 279-293. [https://](https://doi.org/10.1016/0076-6879(94)34095-1) [doi.org/10.1016/0076-6879\(94\)34095-1](https://doi.org/10.1016/0076-6879(94)34095-1)
- Ribeiro, F. A. L., Ferreira, M. M. C., Morano, S. C., da Silva, L. R., & Schneider, R. P. (2008). Planilha de validação: uma nova ferramenta para estimar figuras de mérito na validação de métodos analíticos univariados. *Química Nova*, *31*(1), 164-171. [https://doi.](https://doi.org/10.1590/S0100-40422008000100029) [org/10.1590/S0100-40422008000100029](https://doi.org/10.1590/S0100-40422008000100029)
- Santos, S. D. C., Cruz, K. M. G., Costa, R., Gonçalves, I. M., Lima, R. C. C., Oliveira, S. M., Mello, A. A., Luz, E. R. (2021). Colorimetric determination of iron content in pharmaceutical formulations using a smartphone camera associated with color measurement applications. *Revista Eletrônica Perspectivas da Ciência e Tecnologia*, *13*, 197-207. [https://doi.org/10.22407/1984-5693.2021.](https://doi.org/10.22407/1984-5693.2021.v13.p.197-207) [v13.p.197-207](https://doi.org/10.22407/1984-5693.2021.v13.p.197-207)
- Siddeeg, A., AlKehayez, N. M., Abu-Hiamed, H. A., Al-Sanea, E. A., & AL-Farga, A. M. (2021). Mode of action and determination of antioxidant activity in the dietary sources: An overview. *Saudi Journal of Biological Sciences*, *28*(3), 1633-1644. [https://doi.](https://doi.org/10.1016/j.sjbs.2020.11.064) [org/10.1016/j.sjbs.2020.11.064](https://doi.org/10.1016/j.sjbs.2020.11.064)
- Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *American Journal of Enology and Viticulture*, *16*(3), 144-158. [https://doi.](https://doi.org/10.5344/ajev.1965.16.3.144) [org/10.5344/ajev.1965.16.3.144](https://doi.org/10.5344/ajev.1965.16.3.144)
- Stratil, P., Klejdus, B., & Kubáň, V. (2006). Determination of Total Content of Phenolic Compounds and Their Antioxidant Activity in VegetablesEvaluation of Spectrophotometric Methods. *Journal of Agricultural and Food Chemistry*, *54*(3), 607-616. [https://doi.](https://doi.org/10.1021/jf052334j) [org/10.1021/jf052334j](https://doi.org/10.1021/jf052334j)
- Thongsuk, P., & Sameenoi, Y. (2022). Colorimetric determination of radical scavenging activity of antioxidants using Fe3O4 magnetic

nanoparticles. *Arabian Journal of Chemistry*, *15*(1), 103475. [https://](https://doi.org/10.1016/j.arabjc.2021.103475) doi.org/10.1016/j.arabjc.2021.103475

- Vallejos, S., Moreno, D., Ibeas, S., Muñoz, A., García, F. C., & García, J. M. (2019). Polymeric chemosensor for the colorimetric determination of the total polyphenol index (TPI) in wines. *Food Control*, *106*, 106684.<https://doi.org/10.1016/j.foodcont.2019.06.010>
- Zheng, S., Li, H., Fang, T., Bo, G., Yuan, D., & Ma, J. (2022). Towards citizen science. On-site detection of nitrite and ammonium using a smartphone and social media software. *Science of The Total Environment*, *815*, 152613. [https://doi.org/10.1016/j.](https://doi.org/10.1016/j.scitotenv.2021.152613) [scitotenv.2021.152613](https://doi.org/10.1016/j.scitotenv.2021.152613)