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Development of an Analytical Method Using Smartphones for the Assessment of Phenolic Compounds

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Abstract

Phenolic compounds exhibit antioxidant activity, which is of nutritional interest, as it has been associated with the prevention of various diseases. Therefore, the present study aimed to develop a method for quantifying these compounds in various food matrices using the mobile application PhotoMetrix UVC, a portable, fast, simple, and cost-effective colorimetric tool. Initially, an optimization of the application for the Folin-Ciocalteau method was carried out using a 24 experimental design. In the optimization, grape peel flour samples were used. Different cuvettes, the distance between the endoscope and the samples, the intensity of the image capture light, and the mode of acquisition (RGB and multiple channels) were also varied. It was possible to verify that the conditions that yielded the best results were: glass cuvette, 100 lux brightness, a distance of 0.03 cm between the endoscope and the sample, and acquisition mode in multiple channels. Samples of whiskey, coconut water, and grape seed flour were analyzed. The results were compared with the official spectrophotometric analysis method using a digital spectrophotometer. The method's accuracy and limits of quantification and detection were calculated following EURACHEM standards. The accuracies obtained in relation to the traditional method were 93.3% for grape seed flour, 91.6% for whiskey, and 95.1% for coconut water. It was possible to conclude that the developed method showed good accuracy values. Thus, it was successfully validated, with detection and quantification limits corresponding to 7.98 mg/L and 26.61 mg/L, respectively, which are satisfactory limits since the minimum working value was 44 mg/L.

Keywords: PPhenolic compounds; Quantification; Colorimetry; Photometrix UVC; Smartphone; Quick methods; Low cost

Introduction

Numerous epidemiological studies have demonstrated a strong link between a high consumption of fruits and vegetables and a reduced risk of non-communicable chronic diseases (NTCDs) such as cardiovascular diseases, cancer, obesity, diabetes, and more. This association is attributed to the presence of various antioxidant phytochemicals, in which phenolic compounds play a pivotal role in delivering these potential health benefits [8]. Phenolic compounds are characterized by having aromatic rings and hydroxyl groups in their chemical structure. These functional groups confer them with pharmacological activities of great importance, such as antioxidant activity. This antioxidant action is due to the interaction of phenolic compounds with radical species, promoting their inactivation through proton donation [1]. Spectrophotometry is considered one of the relatively simple techniques for quantifying plant phenolics. For many years, the Folin-Denis and Folin-Ciocalteu methods have been the two widely used spectrophotometric assays for measuring total phenolics in plant materials [9,11]. Both techniques rely on a chemical reduction process using reagents that contain tungsten and molybdenum [14]. The result of this reduction, in the presence of phenolic compounds, produces a blue color characterized by a broad light absorption spectrum centered around 760 nm [5].

Affordable imaging devices such as webcams, scanners, and smartphones, which were not initially designed for analytical purposes, have been employed as alternatives to traditional analytical instruments like spectrophotometers. These devices allow for the adaptation of classical analytical methods, including those based on visible-range spectrophotometry or fluorescence, offering a novel and user-friendly approach to analytical applications [6]. Smartphones have attracted attention as analytical tools due to their widespread availability at affordable prices, coupled with their capability for data acquisition, storage, and processing within a single device [7].

The PhotoMetrix application enables colorimetric analysis through the decomposition of digital images acquired by smartphone cameras, which are then processed within the same device, allowing the use of this app for in situ analysis [3]. This app offers two versions: PhotoMetrix Pro®, which analyzes images taken with the device's camera, and the latest iteration, PhotoMetrix UVC, which supports external camera usage connected to a cellphone for image acquisition. Both versions allow for univariate and multivariate calibration, greatly expanding their applicability in chemical analysis [2].

Therefore, the aim of the present study was to develop a colorimetric analytical method that utilizes the mobile application PhotoMetrix UVC as an analysis tool for the quantification of phenolic compounds. These alternative methods have the characteristic of being fast, simple, cost-effective, and having a high analytical frequency. They also offer applicability for field use as they are portable.

Materials and Methods

Sample Preparation

The food matrices chosen for the development and application of the new methodology for the determination of phenolic compounds were grape peel and grape seed flours, coconut water, and whisky. The grape flours required an extraction process to isolate the phenolic compounds from these matrices. The methodology described by Rodrigues and colleagues [12] was followed for this step. Weighed 0.5 g of grape peel and grape seed flour samples in centrifuge tubes. Then, 10 mL of a methanol: water solution (80:20, v/v, acidified with 0.35% formic acid) was added, and the mixture was vortexed for 3 minutes. The tube contents were centrifuged (high-speed refrigerated centrifuge HITACHI GIII series) at 25000g for 5 minutes at a temperature of 4°C, and the supernatant was removed. To obtain the extract, this procedure was repeated until exhaustive extraction of phenolic compounds. The end of the process was assessed by reacting the obtained supernatants with the Folin-Ciocalteau reagent, with the absence of blue color product formation indicating that the extraction was completed.

Experimental Design for Method Optimization

The method optimization for the phenolic compounds determination was carried out according to a 24 experimental design (Table 1). Sixteen experiments were conducted, varying the following factors: total brightness (100 and 80 lux) determined by the box + endoscope, cuvette type (glass and plastic), distance from the endoscope to the sample (0.05 and 0.03 cm), and image acquisition mode (RGB and Multichannel). A scheme to the experimental setup employedis shown in Figure 1. For these experiments, grape peel flour was used as the sample. The selection of the best experimental condition was based on the accuracy obtained in relation to the conventional method. To ensure the repeatability of this accuracy, a sextuplicate of the mentioned experiment was conducted.

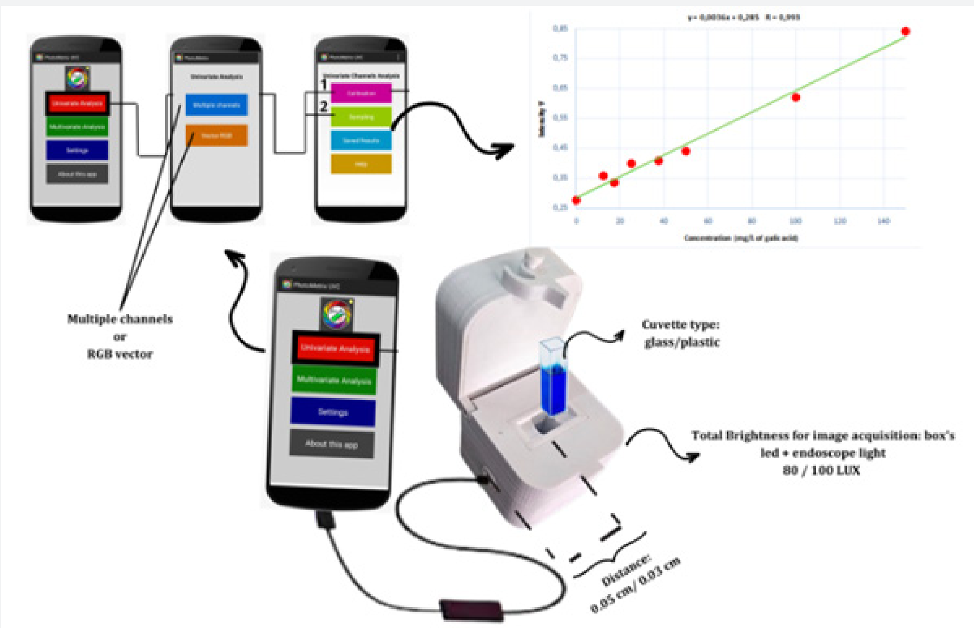

Figure 1: Experimental setup employed to image acquisition with PhotoMetrix UVC.

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Folin-Ciocalteau method

For the method optimization and subsequent validation using the mobile application, the Folin-Ciocalteau method [13] was applied. A 1:2 dilution of the samples was used. In test tubes, 1500 µL of Milli-Q water, 250 µL of each sample, and 250 µL of the Folin-Ciocalteau reagent were added. The tubes were shaken for 20 s. After 5 minutes, 1000 µL of 7% sodium carbonate solution was added. The tubes were shaken for another 20 s. After 2 hours of incubation, readings were taken on the UV-Vis spectrophotometer (SPECTRALE ESU-51) at 765 nm and on the PhotoMetrix UVC. Gallic acid was used as a standard at concentrations of 0, 12.5, 17.5, 25, 37.5, 50, 75, 100, and 150 ppm to construct the calibration curve.

PhotoMetrix UVC analysis

With the optimized method, the samples (grape seed flour, coconut water, and whisky) were analyzed in triplicate following the Folin-Ciocalteau method as mentioned before.In the analysis using the PhotoMetrix UVC application, a Samsung Galaxy J7 Neo smartphone, a B-Max 2M endoscope, and the box obtained through a 3D printer were used, as shown in Figure 1. The procedures to perform the analysis using PhotoMetrix UVC are shown in Figure 2. First, the calibration curve was constructed by taking a picture of each calibration point and informing its concentration to the application. At the end of this process, PhotoMetrix were able to generate the calibration curve, which were used in the "Sampling" step to calculate the concentration of each sample based on their images. The results from the traditional method were obtained by linearcurve fitting followed by interpolation to determine the values for the total phenolic content expressed in mg/L of gallic acid. On the other hand, the PhotoMetrix UVC provided both the calibration curve and the values of phenolic compound concentration, also in mg/L of gallic acid. Both were compared in terms of accuracy calculatedaccording to EURACHEM standards [16] as shown below.

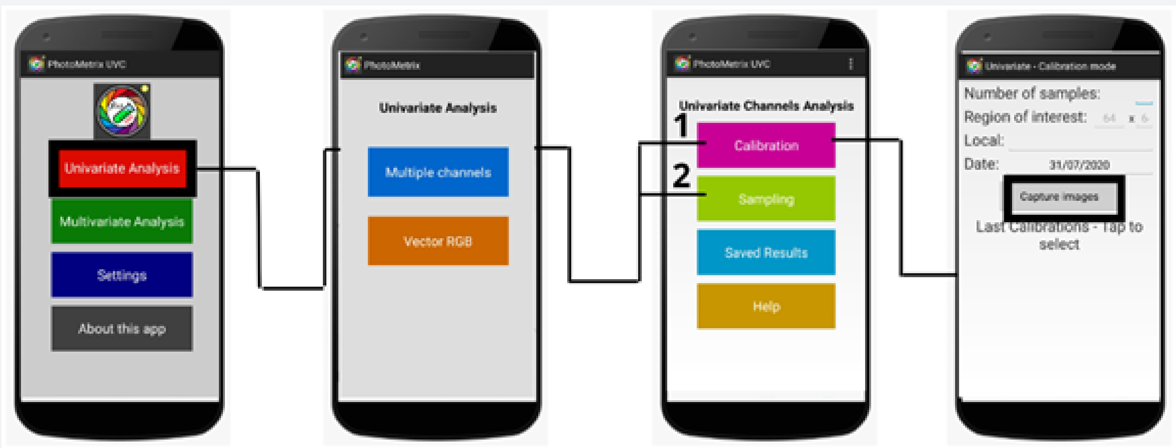

Figure 2: Analisys description on PhotoMetrix UVC.

Quantification and detection limits

To calculate the limits of quantification and detection, 10 tubes with the 'blank' solution were prepared, and readings were taken for each of them using the application, similar to the method described earlier. Based on the concentration results obtained, the LOD and LOQ were calculated according to EURACHEM standards [16] using the equations 1 and 2 below.

$$LOD = 3.So \quad (1)$$

$$LOQ = 10.So \quad (2)$$

Results

Method Optimization

The results obtained in the optimization stage are shown in Table 2. Results within the range of 90% to 110% accuracy were considered good, with those closest to 100% being ideal. The average concentration value obtained through the traditional method was 214.40 mg/L. However, to calculate the accuracy, PhotoMetrix's concentration value was compared to the result of the traditional method performed on the same day. Given that optimization experiments spanned several days and phenolic compounds are sensitive and prone to degradation, both analyses were conducted simultaneously to ensure a fair comparison between the two methods. Experiments 5, 6, 10, 11, 13, and 15 would be classified as having ideal results. However, experiments 5 and 10 showed a high standard deviation between the values obtained with PhotoMetrix UVC, indicating that with this condition it was not possible to achieve a good precision. Additionally, as the experiments progressed, some important practical issues became apparent. In practice, it was observed that working with long distances from the endoscope to the sample, as

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used in experiments 5, 6, 13, and 15, was not feasible, as the lights from the endoscope interfered with capturing images at greater distances, potentially introducing significant experimental errors. Additionally, as both glass and plastic cuvettes yielded good results, for environmental reasons, it was preferred to use glass cuvettes since disposable plastic cuvettes would need to be discarded after each use. Therefore, the conditions described in experiment 11 were chosen: glass cuvette, total brightness of 100 LUX, distance of 0.03 cm between the endoscope and the cuvette and Multichannel as the acquisition mode. With these conditions it was possible to achieve an accuracy of 101.86% and a correlation coefficient of 0.956.The image acquisition mode that yielded the best results was the Multichannel mode. We believe this is because, as it provides a combination of the R, G, and B vectors, it can take into account external interferences that could lead to errors.

Table 1: Experimental design for method optimization.

Experiment	Cuvette type	Total Brightness (lux)	Distance (cm)	Acquisition Mode
1	Glass	80	0.03	RGB
2	Plastic	80	0.03	RGB
3	Glass	100	0.03	RGB
4	Plastic	100	0.03	RGB
5	Glass	80	0.05	RGB
6	Plastic	80	0.05	RGB
7	Glass	100	0.05	RGB
8	Plastic	100	0.05	RGB
9	Glass	80	0.03	Multichannel
10	Plastic	80	0.03	Multichannel
11	Glass	100	0.03	Multichannel
12	Plastic	100	0.03	Multichannel
13	Glass	80	0.05	Multichannel
14	Plastic	80	0.05	Multichannel
15	Glass	100	0.05	Multichannel
16	Plastic	100	0.05	Multichannel

Method application

In Table 3, the concentration values of phenolic compounds (in mg/L of gallic acid) obtained for the samples through the traditional method and PhotoMetrix UVC are shown, along with their respective accuracy values. These concentrations were calculate using the calibration curve for the traditional espectrophotometric method (Figure 3A) and the calibration curve constructed with the smartphone application (Figure 3B), respectively.

It can be concluded that grape seed flour has the highest amount of phenolic compounds, followed by coconut water and whisky, respectively. Additionally, it can be observed from the accuracy values that the results obtained through PhotoMetrix UVC closely approached those obtained through the traditional method. The ANOVA Tukey test ($p = 0.05$) indicated no significant difference between the concentration values obtained by PhotoMetrix UVC and the traditional spectrophotometric method for both whisky and coconut water. However, for grape seed flour, this statistical test revealed a significant difference between the two methods. This could be attributed to the complexity of grape seed flour matrix, wherein there may be certain interferents that absorbed light in the spectrophotometer but were not detected by PhotoMetrix. In addition, suspension solids may cause interference in spectrophotometer readings as well.

In addition, it is possible to verify that the results obtained by the traditional method have a significantly lower standard deviation than those obtained by the PhotoMetrix UVC, indicating that the spectrophotometer is a more accurate tool. On the other hand, it's indeed impressive that a significantly simpler and accessible tool can yield results that are very similar to those of a complex instrument like a spectrophotometer. Analyzing the calibration curves, it's possible to verify that both generated by the traditional method and PhotoMetrix UVC have a correlation coefficient close to 1, indicating a linear relation between absorbance and the concentration of phenolic compounds, as expected. The slope of the calibration curve obtained with the traditional method is higher than that yielded by PhotoMetrix UVC, indicating a more sensitive method. However, both slopes are in the same order of magnitude, which is indeed a good result. Thus, the use of the application for the analysis of phenolic compounds

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has proven to be a viable and promising alternative, depending on the required sensitivity and accuracy.

Quantification and detection limits

The method exhibited LOD = 7.98 mg/L and LOQ = 26.61 mg/L. These results demonstrate that, for the range in which the quantification of phenolic compounds was required, the application met the need, as it has the capability to quantify at these concentrations.

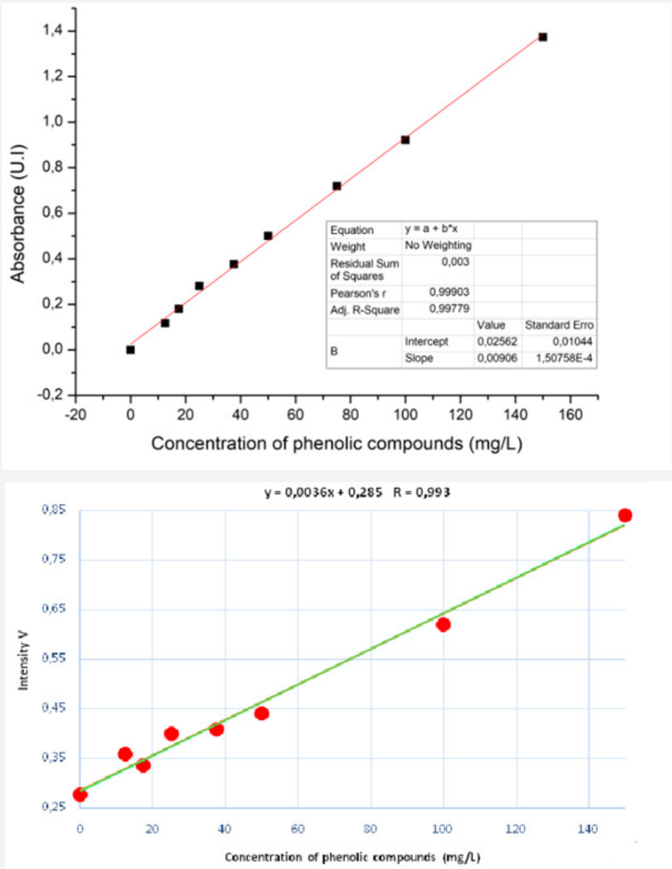

Figure 3: Calibrations curves obtained with traditional espectrophotometric method (A) and with PhotoMetrix UVC (B).

Discussion

The use of alternative methods in chemical analysis, such as those employing smartphones as analytical tools, plays a crucial role in various fields of science and industry. These methods offer accessibility, portability for on-site analyses, user-friendliness, and rapid results, making them valuable for quick decision-making. They also contribute to environmental sustainability by reducing chemical waste and have the potential to democratize access to chemical analysis, significantly impacting scientific research, industry, and decision-making in diverse fields. The study reported in this paper was an example of the application of these alternative methods to determine a group of highly important compounds. It was possible to verify that even though a smartphone is a simple instrument, it can be used for chemical analysis, yielding satisfactory results that were very close to those obtained by a spectrophotometer. The results obtained for coconut water using PhotoMetrix UVC was similar to that one obtained by Tanqueco et al. [15], for the stored product (the same kind used in this paper).The authors determined total phenolics content in stored coconut water of 3 varieties of coconuts namely Laguna Tall (LT), San Ramon Tall (SRT), and Aromatic Dwarf (AD) at different stages of maturity (13-23 months) using the Folin-Ciocalteau method. For the Laguna Tall (LT) and San Ramon Tall (SRT) coconut water they found a concentration of total phenolic compounds equal to 170.70 ± 0.66 mg/1000g and 167.20±0.16 mg/1000g with 18 months of maturity.

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Table 2: Results obtained in the method optimization step.

PhotoMetrix UVC			
Experiment	**Phenolic concentration (mg/L)**	**Correlation coefficient (R)**	**Accuracy**
1	164.77 ± 3.43	0.935	79.79%
2	181.07 ± 5.96	0.981	78.08%
3	217.26 ± 4.69	0.949	90.60%
4	185.36 ± 2.76	0.926	77.30%
5	200.09 ± 14.54	0.865	96.89%
6	205.41 ± 6.13	0.814	99.47%
7	142.81 ± 2.95	0.902	88.69%
8	166.23 ± 6.53	0.882	80.50%
9	185.06 ± 0.00	0.912	89.62%
10	237.26 ± 9.89	0.973	102.31%
11	244.26 ± 5.71	0.956	101.86%
12	220.99 ± 2.51	0.969	92.15%
13	206.99 ± 0.00	0.91	100.23%
14	279.50 ± 3.20	0.867	135.34%
15	163.76 ± 0.00	0.9	101.71%
16	301.25 ± 0.00	0.93	125.62%

Table 3: Phenolic compounds concentration in the analyzed samples obtained by traditional method and PhotoMetrix UVC. Equal letters in the same row mean there's no significant difference between the concentration values at a 95% confidence level. Different letters indicate a significant difference between them with 95% confidence.

	Traditional method	**PhotoMetrix UVC**	
Sample	**Phenolic concentration (mg/L)**		**Accuracy**
Grape seed flour	283.36 ± 0.23a	264.46 ± 5.52b	93.30%
Whisky	150.89 ±2.46a	138.25 ± 8.68a	91.60%
Coconut water	160.00 ± 0.67a	152.15 ± 7.70a	95.10%

PhotoMetrix has been successfully applied in numerous chemical analyses, serving as an alternative to the UV-VIS reference method, consistently delivering satisfactory results. In a study by Böck et al. [4], ethanol content in sugarcane spirit was determined through digital image analysis with a smartphone using PhotoMetrix® in a multivariate PLS calibration model. The results obtained through the mobile phone were compared with those from the UV-VIS method, the reference technique. The model achieved a satisfactory RMSEP of 0.0677%, considering the ethanol concentration range in cachaça falls between 38% and 48%. Furthermore, statistical tests confirmed that there was no significant difference between the results obtained through the proposed and reference methods.

Furthermore, Lumbaque et al. [10] introduced a method for determining dissolved iron and hydrogen peroxide in solar photo-Fenton processes via digital image analysis. They employed two iron determination methods, one using 1,10-phenanthroline and the other with hydroquinone, while hydrogen peroxide was assessed using ammonium metavanadate. Both methods utilized the PhotoMetrix app with multivariate analysis (PLS) and were compared to UV-VIS. The results from the PhotoMetrix app closely mirrored those of the reference method.

Conclusion

It can be concluded that phenolic compounds can be quantified in various food matrices using the PhotoMetrix UVC mobile app, representing a simpler, more portable, and cost-effective detection method with higher analytical frequency. Moreover, this method demonstrates good accuracy when compared to conventional techniques and provides suitable quantification and detection limits within the concentration range under investigation. This approach not only offers a practical alternative for phenolic compound analysis but also enhances accessibility and affordability in analytical processes. This study can serve as a starting point for the implementation of innovative analytical methodologies in the field of food science, fostering significant advancements in the field.

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