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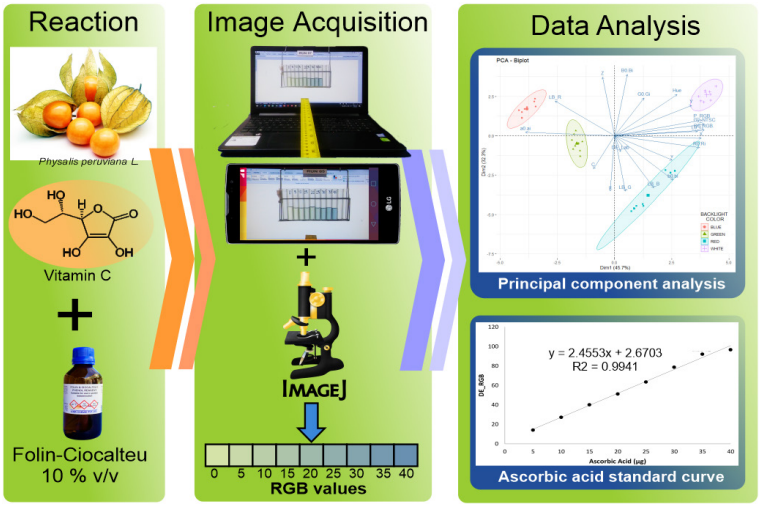
ARTICLE

Use of Smartphone and Image Analysis in the Quantification of Vitamin C in Golden Berry (*Physalis peruviana* L.) Juice

Wilber Vilcapoma Quispe*, Julio Fernando Pérez Sáez

Universidad Nacional de San Cristóbal de Huamanga, Escuela Profesional de Ingeniería en Industrias Alimentarias. Portal Independencia N° 57, Ayacucho, Peru



Conventional methods used to quantify vitamin C require expensive equipment; however, the image analysis method has proven to be effective in quantifying various bioactive compounds and could be useful for small industries due to its low cost. In this sense, the objective of the present work was to evaluate the use of a Smartphone and image analysis in the quantification of vitamin C in golden berries juice. Calibration curves were elaborated with ascorbic acid standards (2.5 – 20 mg L^{-1}) and the Folin-Ciocalteu chromophore reagent (10%). Fifteen color parameters (analytical responses) were obtained from images obtained with a smartphone and the ImageJ program of the colored samples using four backlight colors, to which a principal component analysis was applied using the integrated development environment for R, RStudio. Subsequently, one-way ANOVA and mean comparisons by Tukey's method (α = 0.05) were applied to the best-scoring analytical responses. Ultimately, the quantification of vitamin C in golden berry juice was performed using the image analysis method, which exhibited superior linearity and sensitivity (R^2 = 0.9941 and m = 4.91). A comparative assessment was conducted against a spectrophotometric method utilizing the *t*-Student test for independent samples (α = 0.05), demonstrating no statistically significant difference between the two methods (p > 0.05).

Keywords: Folin-Ciocalteu, ImageJ, RGB model, low-cost analysis, principal component analysis (PCA)

INTRODUCTION

Vitamin C (VC) is an important antioxidant and should be consumed daily between 75-90 mg to maintain healthy blood vessels, skin, teeth, bones, and cartilage.[1] It is also essential in anti-allergic treatments, strengthens the immune system, and prevents flu and infections.[2] In this sense, the golden berries fruits

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(*Physalis peruviana* L.) contain an important amount of VC ranging from 23.3 to 46.0 mg per 100 g of berries.[3–5] Additionally, there are exotic fruits that contain significant amounts of VC, which can be assessed by on-site investigations with portable and low-cost methods of analysis.[6]

On the other hand, one of the methods that are gaining momentum in the analysis and determination of biochemical compounds is the use of digital image analysis.[7] These methods proved to be effective and fast, efficient, and low-cost.[8] These methods have also demonstrated high sensitivity and good linearity based on the slope value and coefficient of determination (R^2) of the calibration curve in different studies.[7] Similarly, dos Santos et al.[6] demonstrated the possibility of using the method based on image analysis in the environment where it is required, being a portable method.

Nowadays, mobile devices have ceased to be a sumptuary object to become objects of common use with multiple utilities, one of them being the application in the capture of images of chromophore analytical samples.[2,6,7,9,10] Its availability in almost all types of social environments makes it possible to use it in the analysis of bioactive compounds *in situ*, mainly in communities with limited economic resources,[10] even in small companies that do not have sophisticated analysis equipment, where a quick analysis of VC is required.[6]

In this sense, the main objective of the present work was to evaluate the use of a smartphone and image analysis in the quantification of VC in golden berries juice (*Physalis peruviana* L.).

MATERIALS AND METHODS

The golden berries fruits were purchased from the wholesale market “Nery García Zárate” in Ayacucho city, province of Huamanga, department of Ayacucho. Folin-Ciocalteu reagent (Loba Chemie PVT Ltd, India). Ascorbic acid (Sigma Aldrich, Germany). Tartaric acid (Insumos Químicos Perú. Peru). Trichloroacetic acid (Oxford, India).

Vitamin C standard preparation

It was performed according to the methodology of Jagota & Dani[11] with some modifications. 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, and 0.40 mL of ascorbic acid stock solution (100 mg L^{-1}) were taken in test tubes. Subsequently, the samples were supplemented with a tartaric acid solution (200 mg L^{-1}) to a final volume of 2 mL, resulting in concentrations of 2.5, 5.0, 7.5, 10, 12.5, 15.0, 17.5, and 20 mg L^{-1} of VC. In addition, the blank was made by placing 2 mL of tartaric acid solution (200 mg L^{-1}) in a test tube. Then, 0.2 mL of Folin-Ciocalteu’s reagent diluted to 10% (v/v) was added to the nine prepared standards and allowed to react for 10 minutes. The calibration curve was elaborated by linear regression with the absorbance values (λ = 760 nm) and using the colorimetric analytical responses.

Determination of VC in a sample

The determination of VC used the technique described by Jagota & Dani[11] with some modifications. Five units of golden berries fruits were crushed in a mortar and pestle. 2 mL of the golden berries juice and 8 mL of 10% (w/v) trichloroacetic acid were added to a centrifuge tube and then centrifuged (P. Selecta, S240, Spain) at 3000 rpm for 15 min. Subsequently, VC determination was performed with 0.2 mL of the supernatant.

Acquisition and image processing conditions.

The VC standards contained in test tubes were placed on a stand adapted to place the samples in front of a personal computer screen (HP, 250 G8, USA) that provided four backlight colors (BC); these are: white, red, green, and blue (Figure 1). In addition, 50% backlight brightness and 50 cm image acquisition distance were used.

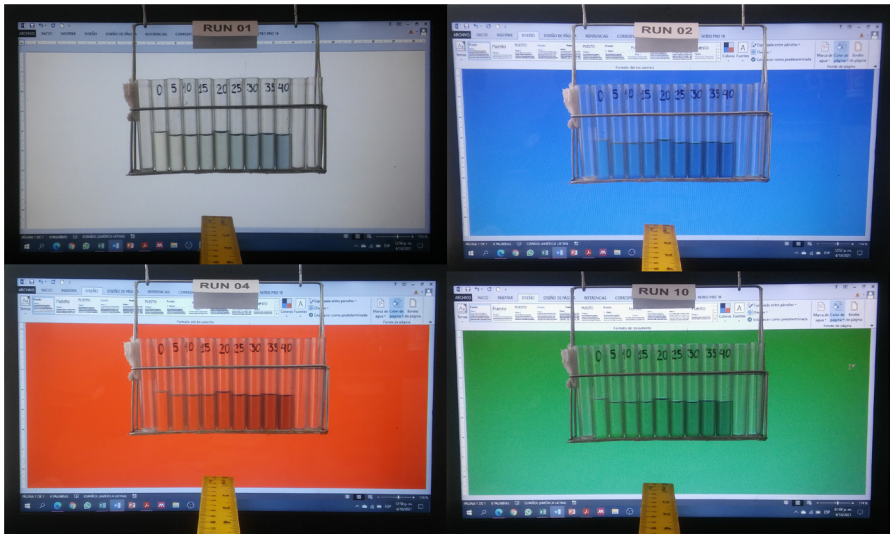

Figure 1. Screens with backlight colors.

Images were acquired with a smartphone camera (LG, H440F, China), 8 MP (Megapixel), and an image acquisition resolution of 3264×2448 pixels. Furthermore, the numerical data of the images, corresponding to the values of the R, G, and B channels of the region of interest, were acquired using ImageJ 1.53k.

Obtaining analytical responses (S)

Different analytical responses (S_1 to S_{15}) were explored by mathematical combinations of the RGB color space and CIE Lab parameters (Table I). Equations (1) to (15) were used for this purpose. The color parameters lightness (L*), redness (a*), and yellowness (b*) were obtained using the worksheet (Microsoft Excel®) developed by Boronkay[12] called Colour Conversion Centre (4.0a), using the RGB channel values.

Table I. Mathematical combinations of the RGB color space and CIE Lab parameters

Analytical responses (S)	Equation	Reference
$S_1 = \frac{R_i + G_i + B_i}{3}$	(1)	Osorio et al.[13]
$S_2 = 0.299R_i + 0.587G_i + 0.114B_i$	(2)	Zhao et al.[14]
$S_3 = -log\left(\frac{R_i}{R_0}\right)$	(3)	Zhao et al.[14]
$S_4 = -log\left(\frac{G_i}{G_0}\right)$	(4)	Zhao et al.[14]
$S_5 = -log\left(\frac{B_i}{B_0}\right)$	(5)	Zhao et al.[14]
$S_6 = R_0 - R_i$	(6)	Porto et al.[2]
$S_7 = G_0 - G_i$	(7)	Porto et al.[2]
$S_8 = B_0 - B_i$	(8)	Porto et al.[2]

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Table I. Mathematical combinations of the RGB color space and CIE Lab parameters (continuation)

Analytical responses (S)	Equation	Reference
$S_9 = \sqrt{(R_0 - R_i)^2 + (G_0 - G_i)^2 + (B_0 - B_i)^2}$	(9)	Abderrahim et al.[15]
$S_{10} = L_0^* - L_i^*$	(10)	Wongthanyakram et al.[16]
$S_{11} = a_0^* - a_i^*$	(11)	Wongthanyakram et al.[16]
$S_{12} = b_0^* - b_i^*$	(12)	Wongthanyakram et al.[16]
$S_{13} = \sqrt{\left(L_0^* - L_i^*\right)^2 + \left(a_0^* - a_i^*\right)^2 + \left(b_0^* - b_i^*\right)^2}$	(13)	Wongthanyakram et al.[16]
$S_{14} = \sqrt{a^{*2} + b^{*2}}$	(14)	Otálora et al.[17]
$S_{15} = \frac{180}{\pi} arctan\left(\frac{b^*}{a^*}\right)$	(15)	Otálora et al.[17]

Red (R), green (G), and blue (B) are color parameters from the RGB color space. The color parameters lightness (L*), redness (a*), and yellowness (b*) from the CIE Lab color space. The subscripts "i" and "0" correspond to the values of the color parameters at different concentrations of vitamin C and without vitamin C, respectively.

Statistical analysis

The statistical software RStudio (version 2022.12.0, Build 353) was employed as the integrated development environment for R (version 4.2.2). The 'stats' library was utilized for the analysis of variance, while the 'EnvStats' library was utilized for obtaining the lack of fit analysis. Multivariate analysis, specifically Principal Component Analysis (PCA), was conducted using the 'missForest', 'FactoMineR', 'ggplot2', and 'factoextra' libraries. Mean comparisons were performed using Tukey's method at a 95% confidence level, with the 'agricolae' library employed for this purpose. The *t*-Student test for independent samples (α = 0.05) was applied using the 'stats' library.

RESULTS AND DISCUSSION

Vitamin C calibration curves (VCCC)

In the realm of analytical chemistry, linear regression has conventionally been employed, although it may not always be the optimal approach. To ascertain the model's suitability and evaluate linearity, it is crucial to assess both the ANOVA and the "lack of fit" value.[18,19] In our study, the models displayed high significance ($p < 0.001$), indicating their linearity and effective utilization as calibration curves. Additionally, the lack of fit value was determined to be non-significant ($p > 0.05$), implying that the experimental data align well with the mathematical model in all three examined cases (Table II).

Table II. *p*-value for the model and lack of fit for different linear models

Source of variation	Red	Green	Blue
Model	< 0.001	< 0.001	< 0.001
Lack of fit	0.9646	0.9700	0.9121

Figure 2 illustrates the concentrations of vitamin C (CVC) and their corresponding analytical responses based on color channels (R, G, and B). Notably, the red channel exhibits more pronounced changes

in the analytical response as the CVC varies, whereas the blue channel demonstrates a comparatively lower response. In both cases, the analytical response shows an inverse relationship with the CVC. This finding aligns with the work of Fan et al.,[7] who emphasized that the color of a sample can be related to its concentration within a specific range, often exhibiting linearity. Consequently, establishing a standard curve enables sample analysis.

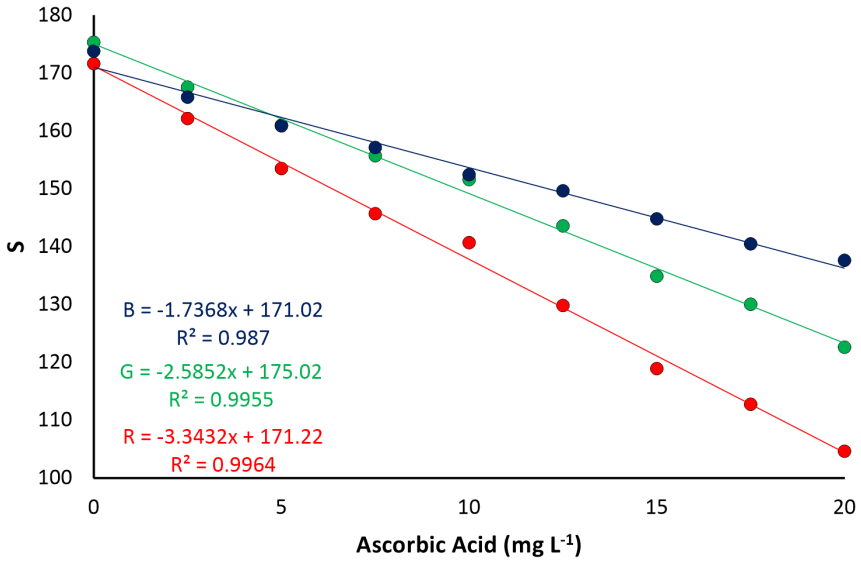

Figure 2. Vitamin C concentration with respect to analytical response (R, G, and B).

Principal component analysis (PCA)

To the fifteen analytical responses (S_1 to S_{15}) shown in Table I, a PCA analysis was applied with respect to the applied background color (BC). The purpose of this analysis was to explore this relationship and its effect on the slope values and the coefficient of determination (R^2) of the VCCC. In this regard, it was observed that certain background colors were associated with certain analytical responses.

The slope values of the VCCC were represented in the PCA-Biplot graph shown in Figure 3-a, and it was observed that BC were grouped in different areas of the graph. In addition, the slope values that had the lowest dispersion were those that worked with white BC. Also, this is located in the first quadrant, which indicated that it contributes significantly to dimension 1 (Dim1), which had a higher contribution value (47.7%). By reducing the variables (Figure 3-b), it was possible to better observe the analytical responses that provided the greatest contribution to increasing slope values. The analytical response's: S_1, S_2, and S_9, had a high positive correlation with respect to Dim1. This is due to the angles formed, these were closer to zero. On the contrary, the S_{11}, had a negative correlation with respect to Dim1, and probably the slope values obtained with this analytical response are lower in relation to the analytical response's found in quadrant 1 of the PCA-Biplot. However, the highest values obtained with this analytical response were with the blue BC. On the other hand, analytical response S_8 did not show a correlation in Dim1, but it did show a correlation in dimension 2 (Dim2). The PCA analysis accounted for 77.9% of the variability in the model, specifically in relation to the slope values of the calibration curve. On the other hand, Attar et al.[20] mentioned that the opposite directions of the arrows indicate inverse correlations between groups of factors. Therefore, the analytical response's that are in the first quadrant provide higher slope values for the white BC and lower values for the blue BC, the inverse happened for the S_{11}. On the other hand, Ballesteros et al.[9] suggested that a higher slope value in a calibration curve would be indicative of a higher sensitivity of the method, which is why the analytical response's "S_1", "S_2", and "S_9" was chosen, in addition to the white BC.

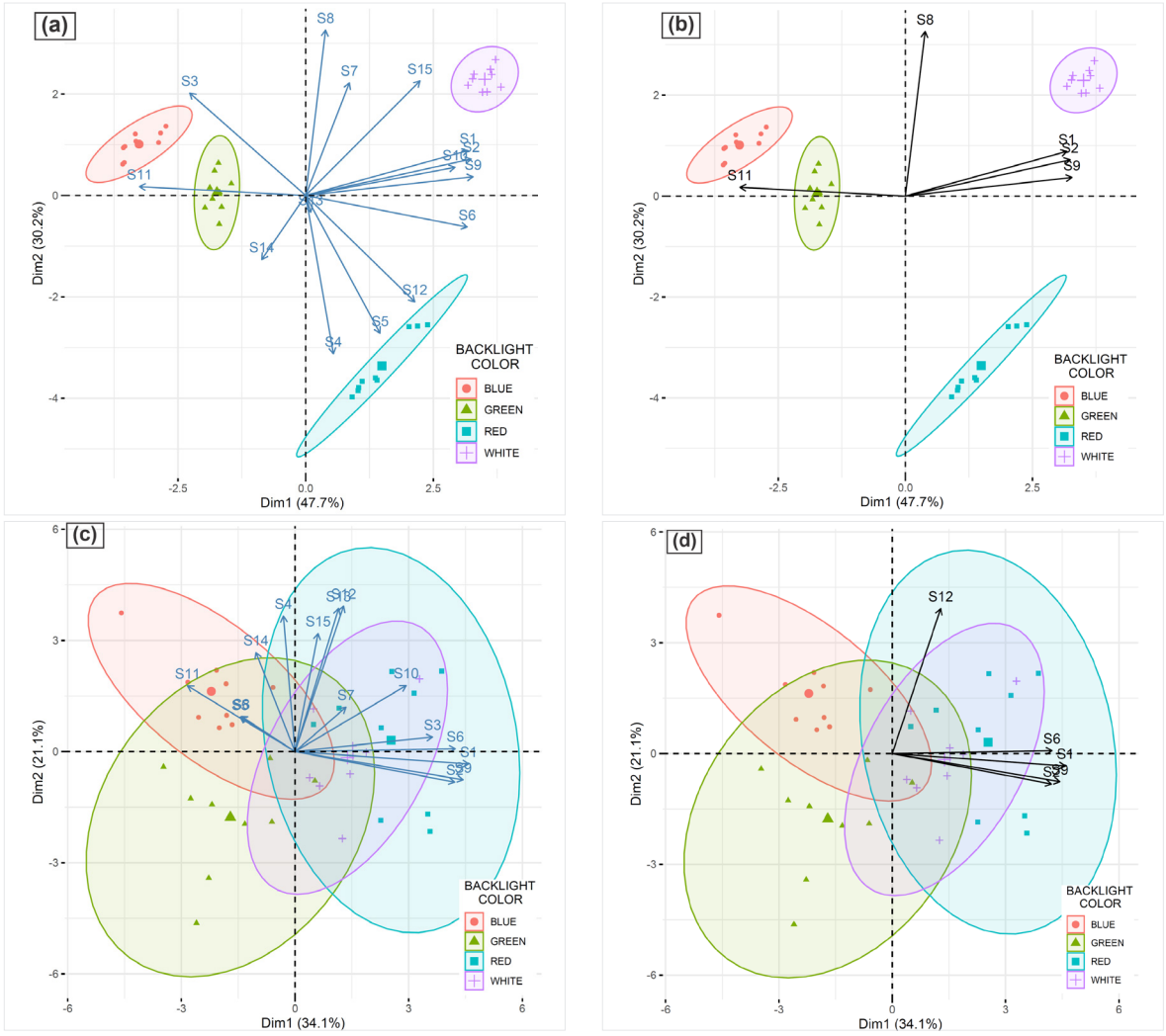

Figure 3. PCA-Biplot in the analysis of the VCCC slope. (a) Complete variables and (b) with variable reduction. PCA-Biplot in the analysis of the R^2 of the VCCC. (c) Complete variables and (d) with variable reduction.

There was no defined group in the analysis of the coefficient of determination with respect to the BC because they appear to form a single group (Figure 3-c). However, the red BC dominates the entire graph, and its center point is on the right, and higher R^2 values can be obtained with this BC. On the other hand, the white BC is in the first quadrant, and its values were not as dispersed as those of the red BC. The analytical response's that contributed the most were S_9, S_1, and S_2 (Figure 3-d). In this second evaluation, the PCA analysis for the R^2 variable explained 55.2% of the model variability. It should be noted that a higher R^2 value would provide greater linearity of method.[21] In addition, Fan et al.[7] analyzed several studies where an R^2 greater than 0.9577 showed good linearity.

After the multivariate analysis, an analysis of mean comparisons was performed using Tukey's method, and evaluated at 95% confidence. Table III shows that the slope value using the analytical response "S_9" was higher and was significantly different ($p < 0.05$) with respect to the analytical response's "S_2" and "S_1". However, when the R^2 was evaluated, none of the analytical response's had significant differences ($p > 0.05$) and their values were above 0.99.

Table III. Comparisons of means between the chosen color parameters

Analytical response's	Slope	R^2
S_9	4.56 ± 0.0772[a]	0.9960 ± 0.0014[a]
S_2	2.72 ± 0.0411[b]	0.9957 ± 0.0012[a]
S_1	2.56 ± 0.0473[c]	0.9960 ± 0.0016[a]

Different lowercase letters (a,b and c) indicate significant differences ($p < 0.05$) in the same column. Values were expressed as mean ± standard deviation (n = 9).

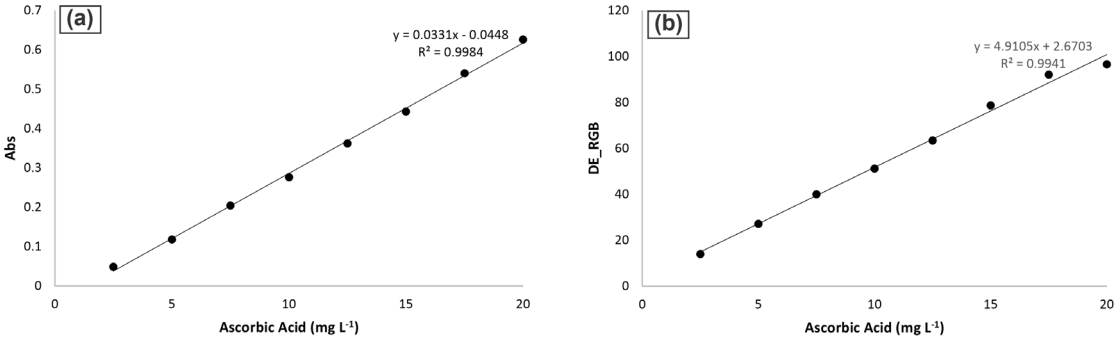

Figure 4. VCCC by spectrophotometric UV-Vis method (a) and image analysis (b).

Analysis of VC in golden berries juice using a smartphone

The VCCC shown in Figure 4 showed high coefficients of determination ($R^2 > 0.99$). According to Fan et al.,[7] this result means that the linearity of the method is very good. On the other hand, the VCCC obtained with the analytical response S_9 and the white BC gave a slope value of 4.91 (Figure 4-b). The LOD and LOQ values of the new method were 1.28 and 3.87 mg L^{-1} VC, respectively. In this regard, Porto et al.[2] found a LOQ of 5.4 mg L^{-1} VC. In our study, the obtained value was slightly lower. However, based on this result, we can still conclude that the new method could be suitable for the analysis of VC in golden berries juice.

Table IV displays the VC values obtained using both the spectrophotometric and image analysis methods utilizing a smartphone. The analysis of VC using these two methods did not yield any significant differences ($p > 0.05$) when evaluated through the t-Student method for independent samples with a 95% confidence level. As a result, it can be concluded that there is insufficient evidence to support the claim that the VCC values obtained differ significantly between the two analysis methods.

Table IV. Vitamin C according to method of analysis

Methods	mg of vitamin C 100^{-1} mL^{-1}
Spectrophotometric*	34.9 ± 0.682[a]
Image analysis	36.4 ± 1.842[a]

*UV-Vis method (λ = 760 nm). Values expressed as mean ± standard deviation for n = 3. Values with equal letters indicate non-significance between treatments according to the Student's *t*-test for independent samples (α = 0.05).

CONCLUSIONS

The utilization of a smartphone and image analysis for quantifying vitamin C (VC) in golden berries juice (*Physalis peruviana* L.) was successfully evaluated, resulting in a value of 36.4 mg VC 100^{-1} mL^{-1} of juice. The Euclidean distance of RGB (S_9) and white backlight color proved to be the optimal analytical response and backlight color, respectively.

Different backlight colors exhibited variations in method sensitivity. However, linearity remained consistent, with high coefficients of determination observed across all backlight colors. Moreover, when employing various color parameters as analytical responses, the method's sensitivity showed significant variability, with slope values ranging from 2.86×10^{-3} to 4.69.

The quantification of VC via smartphone image analysis demonstrated itself as a fast, simple, and cost-effective tool. It exhibited high accuracy comparable to traditional methods. Moreover, this method holds potential for implementation in higher education institutions located in remote areas, where it can be utilized for teaching a low-cost colorimetric analysis method. Furthermore, it opens avenues for the analysis of VC in other sample types.

Conflicts of interest

The authors declare that there is no conflict of interest/competing interest (financial or not) for this study.

REFERENCES

(1) Choy, C. K.; Benzie, I. F. Vitamin C – Health Professional Fact Sheet. *National Institutes of Health, Office of Dietary Supplements*. 2018. https://ods.od.nih.gov/FACTSHEETS/VITAMINC-HEALTHPROFESSIONAL/ (accessed 2021-08-10).

(2) Porto, I. S. A.; Santos Neto, J. H.; dos Santos, L. O.; Gomes, A. A.; Ferreira, S. L. C. Determination of Ascorbic Acid in Natural Fruit Juices Using Digital Image Colorimetry. *Microchem. J.* **2019**, *149*. https://doi.org/10.1016/j.microc.2019.104031

(3) Silva, M. H. B.; Pinheiro, K.; Pereira, M.; Almeida, N. E.; Graciliano, J.; da Silva, F. J.; da Silva, A. Characterization of Physiological Maturity of *Physalis Peruviana* L. Fruits. *Semin. Agrar.* **2021**, *42* (3), 929–947. https://doi.org/10.5433/1679-0359.2021v42n3p929

(4) Arenas, T. Y.; Díaz, I. S. Capacidad Antioxidante del Aguaymanto (*Physalis Peruviana* L.) en tres presentaciones para el Consumo Humano, Universidad Femenina del Sagrado Corazón, Lima-Perú, 2020. http://hdl.handle.net/20.500.11955/725 (accessed 2021-07-07).

(5) Briones-Labarca, V.; Giovagnoli-Vicuña, C.; Figueroa-Alvarez, P.; Quispe-Fuentes, I.; Pérez-Won, M. Extraction of *β*-Carotene, Vitamin C and Antioxidant Compounds from *Physalis Peruviana* (Cape Gooseberry) assisted by High Hydrostatic Pressure. *Food Nutr. Sci.* **2013**, *04* (08), 109–118. https://doi.org/10.4236/fns.2013.48a014

(6) dos Santos, V. B.; da Silva, E. K. N.; de Oliveira, L. M. A.; Suarez, W. T. 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* **2019**, *285*, 340–346. https://doi.org/10.1016/j.foodchem.2019.01.167

(7) Fan, Y.; Li, J.; Guo, Y.; Xie, L.; Zhang, G. Digital Image Colorimetry on Smartphone for Chemical Analysis: A Review. *Meas. J. Int. Meas. Confed.* **2021**, *171*, 108829. https://doi.org/10.1016/j.measurement.2020.108829

(8) Berlín, M. P.; López, A. *Imágenes Digitales En Las Determinaciones Analíticas: Estudio Del Iluminante.* Zaguan Universidad de Zaragoza Repository, 2015. https://zaguan.unizar.es/record/32301 (accessed 2020-12-14).

(9) Ballesteros, J. I.; Caleja-Ballesteros, H. J. R.; Villena, M. C. Digital Image-Based Method for Iron Detection Using Green Tea (*Camellia sinensis*) Extract as Natural Colorimetric Reagent. *Microchem. J.* **2021**, *160* (Part A), 105652. https://doi.org/10.1016/j.microc.2020.105652

(10) Mohamed, A. A.; Shalaby, A. A. Digital Imaging Devices as Sensors for Iron Determination. *Chem. Food* **2019**, *274*, 360–367. https://doi.org/10.1016/j.foodchem.2018.09.014

(11) Jagota, S. K.; Dani, H. M. A New Colorimetric Technique for the Estimation of Vitamin C Using Folin Phenol Reagent. *Anal. Biochem.* **1982**, *127* (1), 178–182. https://doi.org/10.1016/0003-2697(82)90162-2
(12) Boronkay, G. *Colour Conversion Centre*. http://ccc.orgfree.com/ (accessed 2021-08-08).
(13) Osorio, J. A. C.; Urueña, W. A.; Vargas, J. A. M. Técnicas alternativas para la conversión de imágenes a color a escala de grises en el tratamiento digital de imágenes. *Scientia et Technica* **2011**, *1* (47), 207–212.
(14) Zhao, W.; Cao, P.; Zhu, Y.; Liu, S.; Gao, H.-W.; Huang, C. Rapid detection of Vitamin C content in fruits and vegetables using a digital camera and color reaction. *Quim. Nova* **2020**, *43* (10), 1421–1430. http://dx.doi.org/10.21577/0100-4042.20170642
(15) Abderrahim, M.; Arribas, S. M.; Condezo-Hoyos, L. A. A Novel High-Throughput Image Based Rapid Folin-Ciocalteau Assay for Assessment of Reducing Capacity in Foods. *Talanta* **2016**, *152*, 82–89. https://doi.org/10.1016/j.talanta.2016.01.051
(16) Wongthanyakram, J.; Harfield, A.; Masawat, P. A Smart Device-Based Digital Image Colorimetry for Immediate and Simultaneous Determination of Curcumin in Turmeric. *Comput. Electron. Agric.* **2019**, *166*. https://doi.org/10.1016/j.compag.2019.104981
(17) Otálora, M. C.; Wilches-Torres, A.; Gómez Castaño, J. A. Mucilage from yellow pitahaya (*Selenicereus megalanthus*) fruit peel: Extraction, Proximal Analysis, and Molecular Characterization. *Molecules* **2023**, *28* (2), 786. https://doi.org/10.3390/molecules28020786
(18) de Oliveira, E. C. Critical Metrological Evaluation of Fuel Analyses by Measurement Uncertainty. *Metrol. Meas. Syst.* **2011**, *18* (2), 6. https://doi.org/10.2478/v10178-011-0006-4
(19) Chui, Q. S. H. Uncertainties Related to Linear Calibration Curves: A Case Study for Flame Atomic Absorption Spectrometry. *J. Braz. Chem. Soc.* **2007**, *18* (2), 424–430. https://doi.org/10.1590/S0103-50532007000200027
(20) Attar, Ş. H.; Gündeşli, M. A.; Urün, I.; Kafkas, S.; Kafkas, N. E.; Ercisli, S.; Ge, C.; Mlcek, J.; Adamkova, A. Nutritional Analysis of Red-Purple and White-Fleshed Pitaya (*Hylocereus*) Species. *Molecules* **2022**, *27* (3). https://doi.org/10.3390/MOLECULES27030808
(21) Zumbado, H. *Análisis Químico de Los Alimentos: Métodos Clásicos*. International Union of Pure and Applied Chemistry (IUPAC): Cuba, 2004. https://doi.org/10.1351/goldbook