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FOOD TECNHOLOGY

Smartphone-based rapid and low-cost method for the determination of eugenol content of clove essential oil

Izaura Ceolin dos Santos[1,2] Sandra Kunde Schlesner[1] Débora Piovesan de Moraes[1] Daniele de Freitas Fereira[1] Mônica Voss[1] Sandro Rogério Giacomelli[2] Juliano Smanioto Barin[1*]

[1]Departamento de Tecnologia e Ciência dos Alimentos, Universidade Federal de Santa Maria (UFSM), 97105-900, Santa Maria, RS, Brasil. E-mail: juliano@ufsm.br. *Corresponding author.
[2]Departamento de Ciências Exatas e da Terra, Universidade Regional Integrada do Alto Uruguai e Missões (URI), Frederico Westphalen, RS, Brasil.

ABSTRACT: The determination of eugenol in clove essential oil was performed using a smartphone-operated device, which was used for image capture and processing. The colorimetric reaction with the Folin-Ciocalteu reagent was used, and the lighting conditions were evaluated to capture images directly in 2 mL disposable vessels. The free application PhotoMetrix UVC was used for partial least squares regression calibration with suitable values (R^2 greater than 0.99). The accuracy of the proposed method was compared with traditional methods, such as gas chromatography (GC) and spectrophotometry, and it can be observed that there were no significant differences (Student's t-test ($P > 0.05$), with agreements from 97% to 101%. The smartphone method allowed the evaluation of several samples in a few minutes, with simple analysis steps and easy interpretation of the results. The miniaturized scale allowed the use of small amounts of reagents with minimal waste generation. Therefore, the proposed method can be easily operated in the field and allows the evaluation of the quality of clove essential oil in places of restricted access without the need for a laboratory structure and specialized analysts.
Key words: smartphone, clove, eugenol, digital images.

Método rápido e de baixo custo empregando smartphone para a determinação de eugenol em óleo essencial de cravo

RESUMO: A determinação de eugenol em óleo essencial de cravo-da-índia foi realizada utilizando um dispositivo operado com smartphone para captura e processamento de imagens. Para tal desenvolvimento foi utilizada a reação colorimétrica com o reagente de Folin-Ciocalteu. Foram avaliadas diferentes condições de iluminação para a captura das imagens diretamente em frascos descartáveis de 2 mL. O aplicativo livre PhotoMetrix UVC foi usado para a calibração por regressão de mínimos quadrados parciais com valores adequados (R^2 maior que 0.99). A exatidão do método proposto foi comparada com métodos tradicionais, tais como cromatografia gasosa (CG) e espectrofotometria, sem diferenças significativas (teste t de Student ($P > 0,05$) e com concordâncias entre 97% e 101%. O método com smartphone permitiu a avaliação de várias amostras em poucos minutos, com etapas de análise simples e com fácil interpretação dos resultados. A escala miniaturizada permitiu o uso de pequenas quantidades de reagentes e mínima geração de resíduos. Portanto, o método proposto pode ser facilmente operado em campo e permite avaliar a qualidade de óleo essencial de cravo- da- índia em locais de acesso restrito, sem a necessidade de estrutura laboratorial e analistas especializados.
Palavras-chave: smartphone, cravo-da-índia, eugenol, imagens digitais.

INTRODUCTION

Clove (*Syzygium aromaticum, L.*) is used since antiquity as a condiment and flavoring, as well as for the elaboration of perfumes and aromatic incenses. Eugenol is the main component of clove essential oil, but β-caryophyllene and smaller amounts of other components can be found. It is a phenolic compound that has been suggested as a substitute for synthetic additives due to its antioxidant and antimicrobial characteristics (CHAIEB et al., 2007).

The determination of eugenol content in clove essential oil is usually performed by gas chromatography (GC), a very selective technique widely used in laboratories (CHAIEB et al., 2007). However, GC is time-consuming, requires specific knowledge for operation, and presents a high cost and a dedicated laboratory structure, which impairs its use for analysis in the field (NADEEM et al., 2022).

The search for the portability of analytical methods has been increased to allow simple and fast analysis using low-cost devices that the analyst

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can efficiently operate. (NADEEM et al., 2022) The popularization of smartphones increased the scientific community's interest due to the possibility of using sensors coupled (or those embedded in the hardware, as cameras) to these devices, which overcomes the need for specific equipment for chemical analysis (AZEVEDO et al., 2008; COSTA et al., 2021; HOLKEM et al., 2021; SCHLESNER et al., 2022). For the colorimetric methods based on smartphones, digital images or videos must be collected, stored, and processed using mathematical algorithms based on color spaces such as RGB (red, green, and blue) (SANTOS et al., 2019). Lighting conditions and camera position are critical to ensure suitable results in digital image-based methods (DIB). COSTA et al. (2021) proposed a USB endoscopic camera coupled to a closed 3D printed camera under controlled lighting to standardize the experimental conditions in DIB. A hole was provided to allow the introduction of closed disposable vessels, and a free Android application (PhotoMetrix UVC) was developed for a complete analysis solution to obtain good results for different colorimetric analysis (COSTA et al., 2021; HOLKEM et al., 2021; SCHLESNER et al., 2022). Despite the valuable results, no examples of colorimetric analysis in smartphones were found for the quality control of essential oils by the use of such devices.

Therefore, a novel analytical method was developed for the fast and low-cost determination of eugenol in clove essential oils. A field-portable device operated on a smartphone was enough to provide the analysis out of laboratory facilities. The Folin-Ciocalteu reagent was used, and the reaction time and illumination conditions were evaluated. The results obtained with the proposed method were compared with conventional methods, such as spectrophotometry and gas chromatography.

MATERIALS AND METHODS

Samples and reagents

Six samples of clove (*Syzygium aromaticum, L.*) were obtained in the local market of Caiçara-RS, Brazil. The essential oils were extracted from 100 g of clove and 500 mL of ultrapure water (Direct-Q 3 UV, 18.2 MΩcm, Millipore Corp., USA) by hydrodistillation in a Clevenger apparatus for 4 h, according to Brazilian Pharmacopoeia (BRAZIL, 2005). After extraction, the essential oil was collected, dried with anhydrous sodium sulfate (99.5% P.A., Vetec, Brazil), and stored at -18 ºC until analysis. Eugenol (99% P.A., Sigma-Aldrich, Germany) was used to prepare analytical curves in methanol (99.8%, P.A., Sigma-Aldrich, Germany). For the colorimetric assay, 2N Folin-Ciocalteu (Sigma-Aldrich, Germany) and sodium carbonate (99.5% P.A., Sigma-Aldrich, Germany) solutions were used, as well as methanol. The reaction was also evaluated for other compounds reported in the essential oil as β-caryophyllene (≥ 80% P.A., Sigma-Aldrich, Germany), eugenyl acetate (98% P.A., Sigma-Aldrich, Germany), and caryophyllene oxide (95% P.A., Sigma-Aldrich, Germany).

Determination of eugenol by spectrophotometry

The eugenol content of essential oils was determined by the colorimetric reaction between eugenol and the Folin-Ciocalteu reagent (SINGLETON et al., 1999). Calibration curves were constructed with reference solutions of eugenol in the concentration range of 10 to 110 µg/mL prepared in methanol. An aliquot of 0.5 mL of sample (diluted for 0.05 mg of essential oil per mL of methanol) or standards was mixed with 2.5 mL of Folin-Ciocalteu reagent. After 5 min, 2 mL of sodium carbonate 7.5% (w/v) was added, followed by heating at 50 ºC for 5 min to complete the reaction. For analysis by spectrophotometry was used a UV-Vis spectrophotometer (JENWAY UV- 6300 Jenway, UK), and the absorbances were measured at 760 nm (n=3).

Determination of eugenol by smartphone

The colorimetric reaction to determine the eugenol content in clove essential oil was the same used for the spectrophotometry method, as shown in figure 1. For reading in the smartphone (Galaxi J7 prime model), an endoscopic camera (SmartCam, Intelligent Endoscope model, 640 × 480 pixels) was used. The camera was adapted inside a 3D-printed chamber containing a white LED lamp as proposed by COSTA et al. (2021). A hole in the center of the piece was used to introduce a transparent polypropylene vessel (Eppendorf-type, 2 mL, Cralplast, Brazil). Images with a region of interest (ROI) of 64 × 64 pixels were captured and processed with the free application Photometrix UVC version 1.0.7 (GHELFER.NET Inc.), available in the Google Play store. The PLS regression multivariate calibration mode was selected in the applicative, using RGB histogram values (SCHLESNER et al., 2022).

The reaction time was evaluated from 0 to 7 min, and lighting from 19 to 1040 lux. The concentration of eugenol was determined from an analytical curve prepared with eugenol reference solutions from 10 to 110 µg/mL in methanol. All samples were analyzed in triplicate (n=3). The selectivity of the reaction for eugenol was evaluated using reference solutions of beta-caryophyllene,

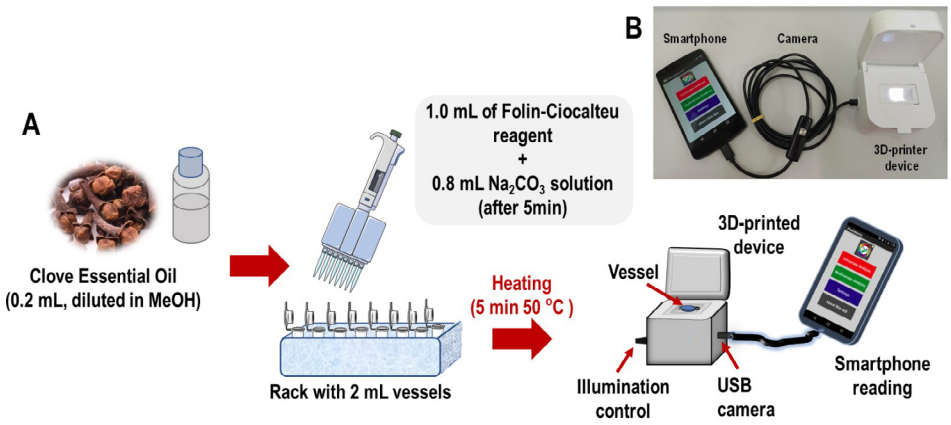

Figure 1 - Procedure used to determine the eugenol content in clove essential oil (A) using the smartphone device (B).

caryophyllene oxide, and eugenyl acetate in the range of 10 to 110 µg/ml. Changes of color were not observed in such concentrations (not shown); and therefore, eugenol was the unique compound that generated colored solutions through the reaction with the Folin-Ciocalteu reagent.

Gas chromatography analysis

For quantification of eugenol by gas chromatography, equipment with a flame ionization detector (GC-FID - Varian Star 3400CX, Palo Alto, CA, USA) was used with a BPX5 fused silica capillary column (25 m × 0.22 mm × 0.25 µm, SGE, Australia). The injection of 1 µL of sample diluted in hexane (1:100 v/v) was introduced into the injector at 230 °C in 1:50 split mode. For the separation of compounds, a heating program was used, starting at 45 °C for 2 min, increasing to 70 °C under a ramp of 10 °C/min. Subsequently, a ramp of 3 °C/min was applied until it reached 200 °C, remaining for 5 min. The gas used was hydrogen at an initial flow rate of 2 mL/min and constant pressure of 15 psi. The temperature of the detector was maintained at 250 °C. The concentration of eugenol in the essential oil was determined from a calibration curve (n=3).

RESULTS AND DISCUSSION

Evaluation of operational parameters in the smartphone-based method

The reaction time was evaluated under heating at 50 ºC (data not shown), and 5 min provided results without a difference from the standard method (SINGLETON et al., 1999), which is performed by 120 min without heating. Thus, 5 min at 50 ºC was used for further experiments.

The illumination was evaluated at different intensities (from 19 to 1040 lux, Figure 2), and influenced the response at the different color channels. For higher light intensities, saturation was observed mainly for the lower concentrations of eugenol. However, a suitable variation for the RGB channels was observed under 75 lux for the different concentrations of eugenol. Therefore, 75 lux was chosen for the subsequent experiments.

Results from the PLS models to determine eugenol in clove essential oil are summarized in table 1. The regression models were developed with 24 samples for calibration. The most suitable results concerning calibration (RMSEC) and cross-validation (RMSECV) errors were obtained with 3 factors, presenting values less than 0.011 and 0.033 mg/mL, respectively.

Determination of eugenol in clove essential oil

According to Figure 3A, a suitable correlation between the measured values errors and predicted values for eugenol content in the development of the calibration model (reference solutions from 10 to 110 µg/mL. The coefficient of determination (R^2 Cal) for all models was higher than 0.99, and no significant differences (ANOVA, $P > 0.05$) among the measured and predicted values were identified.

The determination of eugenol in clove essential oil using a smartphone-based method presented the agreement to the GC and spectrophotometric methods ranging from 97% to 101% (Figure 3B), without a statistical difference (Student's *t*-test, $P > 0.05$). Therefore, using a low-cost device, the proposed method can be considered

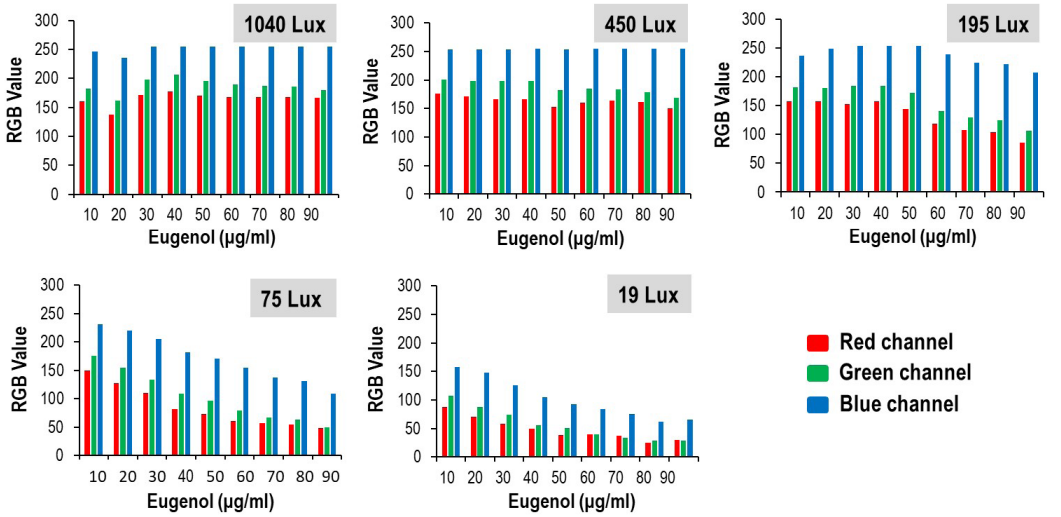

Figure 2 - Evaluation of lighting influence on the RGB values of images for different concentrations of eugenol.

for rapid field-portable analysis. The miniaturization and the low amount of reagents (and residues generated) were relevant features to consider the method environmentally friendly. In addition, only simple sample preparation steps were required, and all data treatment could be performed on the smartphone, which allowed the use of the method by non-specialized analysts.

CONCLUSION

The proposed smartphone-based method allowed the colorimetric determination of eugenol in clove essential oils in an easy-to-use way. Comparable results (agreements ranging from 97% to 101%) were obtained with spectrophotometric and GC methods. In addition to simplicity and low cost, the proposed method was not influenced by ambient lighting and the distance to the camera as commonly found in the colorimetric methods using smartphones. It is important to mention that the analytical steps can be performed in a few minutes, and a straightforward interpretation of data can be obtained using application in the smartphone. In addition, the portability and the evaluation of clove essential oil in hard-to-reach locations were important features of the proposed

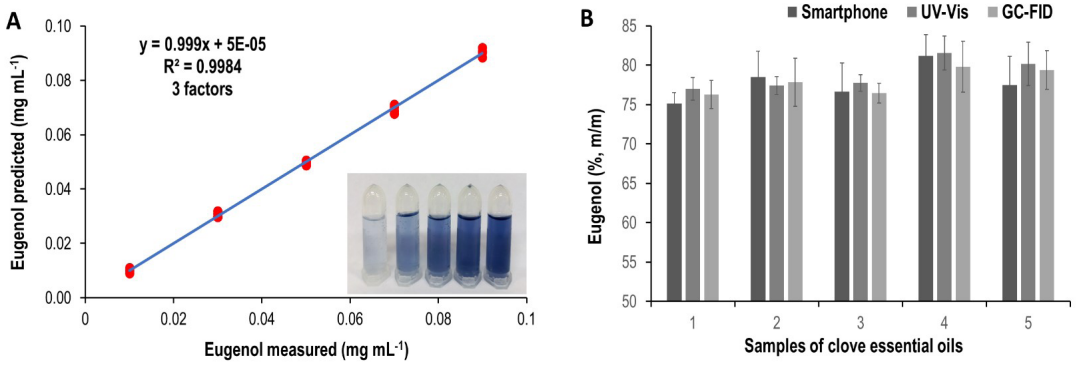

Figure 3 - Results for A) Correlation between eugenol content measured and predicted for known concentrations of eugenol using the proposed smartphone-based method; B) Content of eugenol found in clove essential oil samples obtained by the proposed smartphone-based method, as well spectrophotometry (UV-Vis) and gas chromatography (GC-FID).

Table 1 - Partial least squares regression results for the smartphone-based method for determining eugenol in clove essential oil.

Parameter	Values
Calibration samples	24
Samples prediction	12
Number of factors	3
Slope	0.999
Offset	0.00005
R^2 Cal	0.9984
RMSEC (mg/mL)	0.011203
RMSECV (mg/mL)	0.032607
Bias (mg/mL)	0.0069 – 0.0119
RMSEP (mg/mL)	0.0156 – 0.0351

method because no laboratory facility and specialized equipment were required.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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