Original scientific paper
UDC 665.3:547.56]:621.395.721.5

DETERMINATION OF TOTAL PHENOLIC CONTENT IN VEGETABLE OILS BY SMARTPHONE-BASED IMAGE ANALYSIS

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Abstract

In analytical chemistry, one of the most used methods for the determination of chemical substances in colored solutions is spectroscopy. Spectroscopy is a classical method used for quite some time, but by the development of the smart digital world, there is a possibility to replace classical analytical methods with alternative. One of these alternatives is smartphones-based image analysis on Android or iOS operating systems with god resolution camera systems. The human eyes each wavelength of the visible light can see as a distinctive color. Very important for visible light is the three primary colors, red (R), green (G) and blue (B), which is known as the RGB color model. The aim of the study was determinate the content of TPC with smartphone-based image analysis.

As smartphone for colorimetric detection is used Huawei P30 lite. Android application „ColorPicker", with image matching algorithm, were used for detection of total phenolic content (TPC) in vegetable oils. Total phenolic content was expressed as mg x g^{-1} gallic acid equivalent, and measured by the Folin-Ciocalteu reagent method. For the data comparison and accuracy, spectrophotometer as analytical optical instrument was used. Eleven vegetable oils: sea buckthorn, sunflower, rice, macadamia nut, hemp, corn, grape, linseed, rapeseed, olive and milk thistle were selected for analysis.

Calibration solution show very good coefficient of determination for gallic acid respectively, for red color (R) = R^2 = 0.9954, green color (G) = R^2 = 0.9934 and blue color (B) =R^2 = 0.9713. Red and Blue transmitted light have the same or closest coefficient of determination like by classical spectroscopy R^2 = 0.9997. Results showed that analyses have difference compared with a spectrophotometer in range only from ± 1.7 to 1.2 mg GAE kg^{-1} oil. The highest concentration of total phenolic content in vegetable oils with smartphone was determinate in hemp and olive oil 182 ± 0.2; 178.7 ± 0.2 mg GAE kg^{-1} oil, the lowest by rice, grape and macadamia nut oils, respectively 13 ± 0.1, 13.2 ± 0.1 and 12.8 ± 0.1 mg GAE kg^{-1} oil.

Smartphone-based image analysis is suitable for use in determination of the total phenolic content in vegetable oils.

***Key words**: Smartphone, Digital image analysis, Total phenolic content, Vegetable oils, Android.*

1. Introduction

Vegetable oils or triglycerides are nitrogen-free organic substances that are formed in the metabolism of plants and animals and have a high energy value. Oils are mainly complex mixtures of triglycerides, and different acids may be linked in one triglyceride. Fats and oils are esters of propane-1.2.3-triol (glycerol) and fatty acids [1, 2]. Nutritionists believe that a person's diet needs 30% fat. Fat protects tissues, muscles and internal organs from various shocks, as well as regulates the balanced supply of heat to the body [3]. In addition to fatty acids in vegetable oils, biologically active compounds such as phenolic compounds also play an important role in human health and have influence on the sensory properties and stability of lipid oxidation [4]. Methods for the determination of total phenols with Folin-Ciocalteu reagent are used for the determination of phenolic compounds [5]. The analytical equipment widely used for the determination of total phenols is the spectrophotometer. Use of a spectrophotometer is a classic method, but with the development of the digital world, it is possible to replace it with alternative methods, such as smart devices. One such perspective for smart devices is smartphones, which are already widely used in several fields of science: chemical analysis [6 - 10], paper-based strips [11], Point-of-Care technologies [12], biosensors [13, 14], fruit ripeness [15], food allergens [16, 17] and dermatology [18]. Smartphones are equipped with good resolution cameras and Android operating system, in which the software algorithm can process and absorb the color of light according to Beer-Lambert's law [19]. The light of visible color depends on the wavelengths that the human eye recognizes as different each. There are three primary colors in nature, red (R), green (G) and blue (B), which together form white. In computer science, the intensity of such three colors is expressed as an RGB model and is measured in pixels from 0 to 255 [20]. The RGB modeling system can be used in smartphone-based calorimetry to determine color. The advantage of the smartphones over spectrometry is its ease of use, mobility and device with low-cost. Android based software algorithm, high-resolution sensitive cameras and sensors allow to determine colored solutions according to Beer-Lambert's law.

The aim of the study was determinate the content of total phenolic content with smartphone-based image analysis.

2. Materials and Methods

2.1 Materials

The principle of the colorimetric analysis of the research object is the digital imaging of vegetable oils prepared for the determination of total phenolic content with smartphone-based software application for color analysis with Red, Green, Blue (RGB) color model.

2.1.1 Samples

In total, eleven vegetable oils: sea buckthorn, sunflower, rice, macadamia nut, hemp, corn, grape, linseed, rapeseed, olive, and milk thistle oils in the original commercial packaging were selected for analysis.

2.1.2 Equipment for analysis

From the equipment for analysis we used: Agilent Cary 60 UV/VIS Spectrophotometer (Agilent Technologies, Inc., US) was used for the comparison with the smartphone Huawei P30 Lite (Huawei Technologies Co., Ltd., China) measurement. For imaging, the smartphone Huawei P30 Lite (Huawei Technologies Co., Ltd., China) released 2019, April 25, operating system EMUI 10 (Android 10), 48-megapixel triple camera.

2.1.3 Image acquisition system

The digital image acquisition system consisted of a polyvinyl chloride PULUZ photo studio (Puluz Technology Ltd., China) softbox (24 x 32 x 38 cm^3). Constant light intensity was provided by a 40 pieces light-emitting diode (LED) lamp (model 2835), with luminous flux 550 lm, colour temperature: 3200 K and power: 3.5 W. LED lamp was located at the upper part inside the lightbox. For taking a colorimetric image, a smartphone with a 48-megapixel camera (Huawei P30 Lite) was positioned outside in front of open side of the box at a distance of 12 cm from the PS 2.5 mL macro disposable cuvettes (BrandTech Scientific, Inc., US) with vegetable oil samples with Folin-Ciocalteu's phenol reagent, gallic acid calibration solutions with Folin-Ciocalteu's phenol reagent or 96% ethanol solution.

2.2. Methods

2.2.1 Imaging and image analysis

Image was captured by the smartphone camera and saved as 8-bit JPG format with the average size of 7.0 (8000 x 6000 pixels), ISO 400, f/1.8, 27 mm (wide), 1/2.0˝, 0.8 µm, PDAF (Phase Detection Autofocus). The image was analyzed by a RGB colour model, application for Android "ColorPicker", which was installed from Android Apps on Google Play store. The image analysis system was used to relate the colour of the sample with Folin-Ciocalteu`s phenol reagent.

2.2.2 Determination of Total Phenolic content (TPC)

The concentration of the total phenolic compounds in the vegetable oils was determined using the Folin-Ciocalteu method with some modification [21].

2.2.3 Calibration of gallic acid

Standard solution of gallic acid (Sigma, China) were prepared with concentration: 1, 2, 3, 5, 10, 20, 40, 80, 160 and 200 mg x L^{-1} in 96% ethanol solution. 0.5 mL of each standard solution were mixed with 2.5 mL of 0.2 M Folin-Ciocalteu's phenol reagent (Sigma, Switzerland), after 5 min. 2 mL of 7.5 % Na_2CO_3 (Sigma-Aldrich Chemie GmbH, Germany) was added. After 2h of incubation at room temperature (21 ± 1 ^{0}C), the absorbance was measured at 760 nm using spectrophotometer and smartphone. As blank for spectrophotometer and smartphone was 96% ethanol solution. Calibration curve of gallic acid (mg GEA L^{-1}) with spectrophotometer showed in Figure 1.

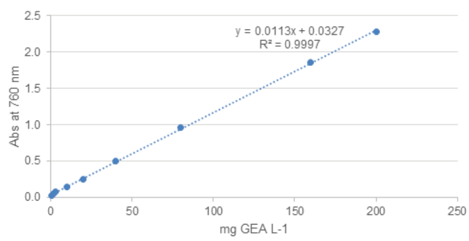


Figure 1. Calibration curve of gallic acid (mg GEA L^{-1}) with spectrophotometer

2.2.4 Determination of TPC in vegetable oils

0.5 g of vegetable oil were mixed with 2.5 mL of 0.2 M Folin-Ciocalteu reagent and vortexed 1 min. with IKA Vortex 3, after 5 min. 2 mL of 7.5 % Na_2CO_3 (Sigma-Aldrich Chemie GmbH, Germany) in 15 mL 120 x 17 mm conical bottom PP tube (Sarstedt AG & Co.KG, Germany), and vortexed 30 sec. with IKA Vortex 3 (KA®-Werke KG, Germany) by speed 7. After 2h of incubation at room temperature (21 ± 1 ^{0}C) the emulsion were centrifuge at 10,000 rpm for 5 min. Coloured solution transfered to the PS 2.5 mL macro disposable cuvettes (BrandTech Scientific, USA) with dimensions 12.5 x 12.5 x 45 mm. Absorbance of the sample against blank (96% ethanol) was measured at 760 nm using UV/VIS spectrophotometer Agilent Cary 60 (Agilent Technologies, USA). Direct imaging of oil samples with Folin-Ciocalteu reagent and 96% ethanol solution in cuvettes were captured using a smartphone-based colorimetric application by RGB colour model according to illustration of Figure 2.

2.2.5 Calculation method for determination of total phenolic content with smartphone-based image analysis

Total phenolic content was calculated using calibration curve for gallic acid and results were expressed as the gallic acid equivalent in mg per kg^{-1} (mg GEA x kg^{-1}). Samples were analysed in tenfold.

2.2.6 Calculation method

Obtained image from smartphone application ColorPicker in RGB mode average color value from the individual red (R_{avg}), green (G_{avg}), and blue (B_{avg}), colors by the equation 1, 2 and 3:

$$R_{avg} = \frac{\sum_{i=1}^{n} R_i}{n} \quad (1)$$

$$G_{avg} = \frac{\sum_{i=1}^{n} G_i}{n} \quad (2)$$

$$B_{avg} = \frac{\sum_{i=1}^{n} B_i}{n} \quad (3)$$

Averaged value of R, G and B converted to absorbance by Beer-Lambert's equation 4:

$$Abs = -\log\left(\frac{I}{I_0}\right) \quad (4)$$

Where:
I - averaged value of R_{avg}, G_{avg} or B_{avg}.
I_0 - averaged value of 96% ethanol solution by RGB from smartphone application.

Calculation of concentration of total phenolic content (TPC) by intensity of Red transmitted light ($C_{R(TPC)}$), equation 5:

$$C_{R(TPC)} = \frac{(Abs_R - 0.0038)}{0.0056} = (\text{mg GEA x kg}^{-1}\text{ oil}) \quad (5)$$

2.2.7 Data processing/statistical analysis

The data of the research was analysed by the statistical and mathematical methods (mean, standard deviation). Data compare by the analysis of variance (ANOVA) and significance was defined at $p < 0.05$. For the data analysis the Microsoft Excel software of the version 2019 was used.

3. Results and Discussion

As demonstrated in Figure 3, is possible to obtain with smartphone good coefficient of determination for calibration solution of gallic acid, for red color (R) = R^2 = 0.9934, green color (G) = R^2 = 0.9938 and blue color (B) = R^2 = 0.9752.

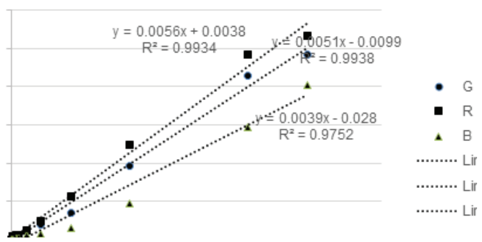
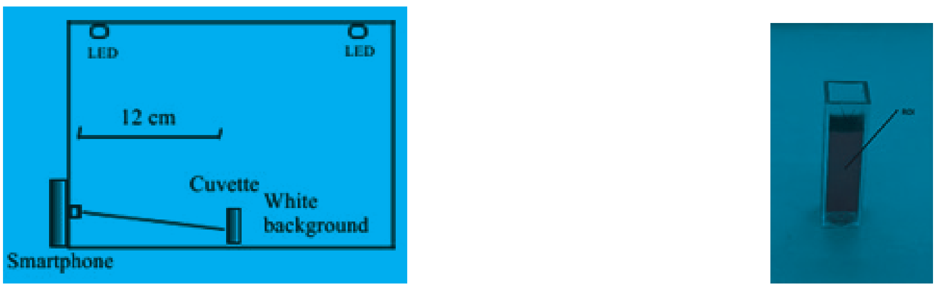


Figure 3. Calibration curve of gallic acid (mg GEA x kg^{-1}) for RGB with smartphone



a.

b.

Figure 2. (a) Illustration of photo studio lightbox experimental setup for image acquisition; (b) example of region of interest (ROI) from a TPC image

Red and Blue transmitted light respectively show the same or closest coefficient of determination like by classical spectroscopy $R^2 = 0.9997$.

Though all RGB model show good coefficient of determination, but by analyses of total phenolic compounds in the vegetable oils, intensity of Red (R) transmitted light is used. In Folin-Ciocalteu method for determination of TPC blue color of solution is formed. Color wheel show that complimentary color of blue is the orange (Figure 4), but only three colors, red (R), green (G) and blue (B) are primary and can direct imaging with android application ColorPicker and therefore for determination of TPC choose a color that closely matches the complementary color or Red (Figure 4).

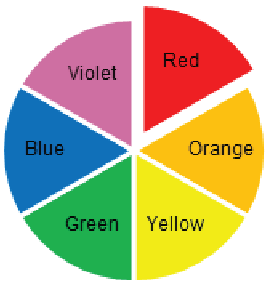


Figure 4. Color wheel of primary and secondary colors

UV/VIS spectrophotometry was used as reference method for comparison with smartphone. Results shows (Table 1) that analyses have difference in range only from ±1.7 to 1.2 mg GAE x kg^{-1} oil.

Table 1. Total phenolic content (TPC), expressed as mg of gallic acid equivalents (GAE) per kg of oil

Vegetable oils	Spectrophotometer	Smartphone by red color transmitted light
	TPC (mg GAE kg^{-1} oil)	
Hemp	181.4 ± 0.2	182.2 ± 0.2
Olive	179.2 ± 0.2	178.7 ± 0.2
Sea buckthorn	105.7 ± 0.2	104.0 ± 0.2
Milk thistle	90.5 ± 0.1	90.3 ± 0.1
Rapeseed	89.5 ± 0.1	88.0 ± 0.1
Linseed	35.7 ± 0.1	36.5 ± 0.1
Sunflower	30.7 ± 0.1	30.2 ± 0.1
Rice	13.4 ± 0.1	13.0 ± 0.1
Corn	13.2 ± 0.1	13.1 ± 0.1
Grape	13.0 ± 0.1	13.2 ± 0.1
Macadamia nut	11.6 ± 0.1	12.8 ± 0.1

Calculated *t*-value for smartphone-based results was lower than the critical (0.47, $p > 0.05$), therefore no statistical difference at a 95% confidence level between the total phenolic content by using the smartphone and UV/Vis spectroscopy. The highest concentration of total phenolic content in vegetable oils was determinate in hemp and olive oil for spectrophotometer 182.9 ± 0.2; 179.2 ± 0.2 mg GAE kg^{-1} oil and smartphone 182 ± 0.2; 178.7 ± 0.2 mg GAE x kg^{-1} oil, the lowest by rice, grape and macadamia nut oils, respectively spectrophotometry 13.4 ± 0., 13.0 ± 0.1, 11.6 ± 0.1 mg GAE x kg^{-1} oil and smartphone 13 ± 0.1, 13.2 ± 0,1 and 12.8 ± 0.1 mg GAE x kg^{-1} oil. Relative standart deviation (%RSD) for UV/Vis and smartphone was the same in range from 0.1 to 0.2. Unfortunately, there are no data in scientific publications on the use of a smartphone to determine the total phenol content.

Acknowledgement

The research was supported by the Latvia University of Life Sciences and Technologies under the project 'Strengthening Research Capacity in the Latvia University of Life Sciences and Technologies'. Project No. 3.2-10/126, Z46.

4. Conclusions

- The research provides a new methodology for determining the total phenol content in vegetable oils with a smartphone-based image analysis. Principle of the method, using a smartphone camera and the Android software that determines the RGB model, allows to determine the total phenolic compounds with the Folin-Ciocalteu's reagent.
- Compared to spectrometry, the smartphone-based colorimetry shows the same results, and its use is much simpler, more mobile and with low cost, therefore, it is necessary to develop the various chemical analyses using smartphone-based image analysis.

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