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Paper-based smartphone chemosensor for reflectometric on-site total polyphenols quantification in olive oil

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Abstract

High polyphenols levels are one of the main quality characters of Extra Virgin Olive Oil (EVOO), leading to high antioxidant activity and contributing to the EVOO health beneficial effects. Analytical methods for the rapid assessment of EVOO polyphenols content, possibly directly at the place of production or distribution, are therefore important for the commercial enhancement of this product. Unfortunately, most analytical methods for quantification of polyphenols in EVOO require laboratory instrumentation and preliminary sample extraction procedures. In this paper we describe a paper-based chemosensor based on the well-known Folin-Ciocalteu colorimetric assay for the rapid (assay time 15 minutes) quantitative detection of polyphenols in EVOO without any preliminary sample extraction. Use of n-propanol for diluting the EVOO sample allows its direct analysis on paper supports functionalized with the Folin-Ciocalteu reagent. The color change of the paper supports is measured using a smartphone camera. A disposable analytical cartridge containing all necessary reagents, including calibration standards, and accessories for performing the assay using a Samsung S8 smartphone have been developed to perform on-site analysis. Measurement of total polyphenol content of EVOO samples gave results in good agreement with the conventional Folin-Ciocalteu assay, and the limit of detection was 30 μg gallic acid equivalents g^{-1} EVOO. The same approach can be employed to measure polyphenols in other oils of vegetal origin.

Keywords

Folin-Ciocalteu assay; polyphenols; smartphone reflectance detection; extra virgin olive oil; chemosensor; 3D printing technology.

1. Introduction

Extra virgin olive oil (EVOO), obtained by mechanical pressing of *Olea europaea* olives, is well-known for its health beneficial effects, which are mainly attributed to the high content of unsaturated fatty acids and phenolic compounds [1]. Indeed, international organizations such as the European Food Safety Authority (EFSA) and the United States Food and Drug Administration (FDA) have approved “health claims” relating regular EVOO consumption to reduced risk of cardiovascular diseases [2, 3]. The main parameters related to EVOO quality are low acidity, low peroxide value, and high antioxidant activity. The polyphenols content of EVOO, which is the main responsible for antioxidant activity, depends on several factors, such as cultivar, growing area, agronomic practices, olive tree diseases, climatic conditions, fruit ripening at harvest, extraction technology, storage duration and conditions. The main phenolic compounds in EVOO are secoiridoids (oleuropein and ligstroside isomers) and their derivatives, such as tyrosol and hydroxytyrosol. These compounds act as free radical scavengers, thus possess a strong antioxidant activity and show preventive action against cardiovascular diseases and anticancer activity [4, 5]. Phenolic compounds play a major role in the overall quality of this highly valuable vegetable oil, contributing not only to EVOO’s beneficial effects [6], but also to its shelf-life [7] and organoleptic features, as they produce a distinctive bitter, astringent and pungent perception [8]. Therefore, rapid, cost-effective, environment-friendly and simple analytical methods for determining phenolic compounds in EVOO on-site are highly desirable for proper product valorisation and characterization of their nutritional added value.

One of the most well-recognized analytical technique for quantifying total phenolic compounds is based on the spectrophotometric analysis after chromogenic reaction given by the Folin-Ciocalteu (FC) reagent. In the presence of phenolic compounds the FC reagent, a mixture of phosphotungstic ($\text{H}_3\text{PW}_{12}\text{O}_{40}$) and phosphomolybdic ($\text{H}_3\text{PMo}_{12}\text{O}_{40}$) acid, is reduced to blue tungsten (W_8O_{23}) and molybdenum (Mo_8O_{23}) oxides [9, 10], which display light absorption around 750 nm. Gallic acid is commonly used as a standard in the FC assay, so assay results are conventionally expressed in gallic acid equivalents (GAE) per unit sample. This assay is widely used because of its simplicity and reproducibility. Nevertheless, it requires laboratory instrumentation and relatively long analysis times, especially when applied to non-aqueous matrices such as EVOO. Indeed, in such case a preliminary sample extraction, usually carried out by liquid-liquid extraction (LLE) or solid-phase extraction (SPE), is required to obtain an aqueous polyphenols solution suitable for performing the assay. Therefore, analyses must be performed in a laboratory environment, with significant costs

and time wasting. In addition, sample extraction is laborious and time consuming and it requires organic solvents such as n-hexane.

The development of portable devices able to directly quantify the total polyphenols content of EVOO without any preliminary sample pre-treatment is attracting great interest in food and nutraceutical industry. To our knowledge, in literature there are only a few examples of portable chemosensors for the detection of polyphenols, which are mainly based on electrochemical detection [11-14]. A microfluidic chip exploiting the luminol-potassium periodate- Fe^{2+} chemiluminescence reaction has been developed for estimating the total phenolic content in olive oil samples, however preliminary sample extraction was required [15].

To enable wide and simple *on-site* application, many chemo- and biosensors exploiting smartphone technology for optical detection have been described [16, 17]. Indeed, optical detection based on imaging of a coloured surface can be easily integrated with smartphone technology taking advantage of the most recent smartphone photocameras based on back-illuminated complementary metal-oxide semiconductor (BI-CMOS) sensors, which have reached performance suitable for employment in analytical methods [18, 19]. Hidayat et al. [20] developed a paper-based sensor for the determination of total phenolic content in green tea beverages based on immobilization on a paper test strip of sodium metaperiodate (NaIO_4) and 3-methyl-2-benzothiazolinone hydrazone (MBTH) as colouring agents. However, the sensor employs toxic reagents and it has not been applied to lipid matrices. Dawan et al. [21] realized another paper-based analytical device for determination of total capsaicinoids in chilli samples exploiting the FC reaction, using a flatbed optical scanner for quantitative evaluation of the reaction products. Also, application to lipid matrices has not been explored.

For the *on-site* analysis of EVOO samples extraction-free methods are desirable. A gold nanoparticles-based colorimetric assay has been recently described for extraction-free determination of total polyphenols in EVOO and other fat-rich samples. In this assay dimethyl sulfoxide (DMSO) was employed as organic solvent for providing suitable environment to bring analytes from the fat-rich sample to the aqueous phase where the nanoparticles-based colorimetric assay took place. However, in this assay spectrophotometric detection required the use of dedicated laboratory instrumentation [22].

In this paper, we describe a paper-based chemosensor exploiting the FC reaction for the quantitative detection of polyphenols in EVOO samples without any preliminary sample extraction. The solvent n-propanol was employed to make EVOO samples compatible with the alcoholic environment in

which the FC reaction takes place. The colour change deriving from the reduction of FC reagent in the presence of phenolic compounds was measured using a smartphone camera. To obtain reproducible and uniform paper support illumination during reflectometry measurement, the smartphone flash was exploited in combination with a light diffusion system. The main advantages of this chemosensor are its ease of use, the elimination of pre-analytical extraction procedures and the fast response (the overall assay time is about 15 minutes). A disposable all-in-one analytical cartridge that contains all necessary reagents was developed in conjunction with accessories for performing the assay using a Samsung S8 smartphone. With this approach, a mobile, stand-alone chemosensor was realized to be used for EVOO characterization directly at the production sites, thus leading to significant reductions in costs and time compared with the current practice requiring laboratory analyses.

2. Materials and Methods

2.1. Chemicals

Sodium carbonate, gallic acid and FC reagent were purchased from Sigma Aldrich (St Louis, MO). Solvents were purchased from Carlo Erba (Milan, Italy) and all other chemicals were of the highest analytical grade. The EVOO samples were supplied by Italian manufacturing companies participating in the VIOLIN research project (Valorization of Italian OLive products through INnovative analytical tools) funded by Cariplo Foundation within the “Agroalimentare e Ricerca” (AGER) program.

2.2. Functionalization of the paper support

For the analysis of EVOO samples, 1 × 1 cm paper supports (Whatman™ No. 1 CHR chromatographic paper, GE Healthcare Life Sciences, Little Chalfont, England) were functionalized by dispensing 5 µL of FC reagent. The supports were left to dry on air, then inserted in the microfluidic system (see below) and stored under vacuum in the dark and at +4°C.

2.3. Polyphenols extraction for microtiter-plate FC assay

For assay validation, polyphenol extracts of EVOO samples were obtained employing a slightly modified literature procedure [23]. Briefly, 1 g of EVOO was dissolved in 1 mL of n-hexane and extracted with 4 × 1 mL of 60:40 (v/v) methanol/water. To remove trace lipids, 2 mL of n-hexane were added to the methanol/water extract, the mixture was then vortexed and centrifuged for 5

min at 3000 rpm. The methanol/water layer was collected, and 2 mL of solution were transferred into a glass tube. Methanol was removed under reduced pressure in an Univapo 150H centrifugal evaporator (UniEquip, Planegg, Germany) and the residual solution was lyophilized using a benchtop lyophilizer (Alpha 1-2 LDplus, Martin Christ GmbH, Osterode am Harz, Germany). The lyophilized extracts were stored at -20°C until analysis. For analysis, the extracts were reconstituted with 250 µL of DMSO.

2.4. Microtiter-plate FC assay

The standard FC assay was performed using a literature procedure [24]. One hundred µL of samples, gallic acid standard solutions in methanol (concentration range 10 – 400 µg mL⁻¹) or blank (methanol) were transferred into 1.5-mL Eppendorf tubes, then 200 µL of FC reagent diluted 1:10 (v/v) in methanol was added to each tube and vortexed thoroughly. Afterwards, 800 µL of a 700 mM aqueous solution of Na₂CO₃ were added to the tubes and the mixtures were incubated at room temperature for 2 h. After incubation, 200 µL of solutions were transferred into the wells of a clear 96-well microplate and the absorbance at 765 nm was measured. The total polyphenols content of the samples, expressed in µg GAE for unit sample, was obtained by interpolation of the absorbance values on the linear calibration curve generated using the readouts of the gallic acid standard solutions.

2.5. Chemosensor

The chemosensor (Figure 1) consisted of four separate components: (a) a microfluidic system, (b) a lab-case enclosing the microfluidic system, (c) a dark box and (d) a smartphone holder.

The microfluidic system (Figure 1A) was fabricated in polydimethylsiloxane (PDMS) exploiting the REplica-Molding (REM) technique. The moulds for REM were designed by the freeware 3D design software SketchUp (Trimble Inc., Sunnyvale, CA) and produced in acrylonitrile-butadiene-styrene (ABS) by Fused Deposition Modelling (FDM) 3D printing using a Replicator 2X Desktop 3D Printer (MakerBot Industries, New York, NY). The microfluidic system contains the functionalized paper supports and the reagents required for performing the analysis. It is organized in three levels, which were separately fabricated and then assembled employing non-polymerized PDMS upon insertion of the functionalized paper supports. The first level contains four 15-µL, non-communicating chambers for the alkaline solution (10% (w/v) Na₂CO₃ solution), as well as venting holes to allow rapid paper drying after the transfer of the alkaline solution on the paper supports. The second level

contains a 20- μ L chamber for loading the EVOO sample, a 180- μ L reservoir for the n-propanol solvent used for sample dilution, two 150- μ L reservoirs for gallic acid standards (rice oil spiked with 250 or 500 μ g g⁻¹ gallic acid and diluted 1:10 (v/v) in n-propanol) and a 150- μ L reservoir for the blank (rice oil diluted 1:10 (v/v) in n-propanol), as well as the fluidic channels for transferring the solutions to the paper supports. The n-propanol solvent reservoir and the EVOO sample loading chamber are connected to the paper support chamber by a channel with a “zig-zag” geometry to assure thorough mixing of EVOO sample and n-propanol before dispensing on paper. The third level consists of four non-communicating chambers, each containing a paper support functionalized with the FC reagent. The other device components were produced in ABS by FDM 3D printing as described above.

The lab-case (125 × 50 × 15 mm), which encloses the PDMS microfluidic system, has a window on the upper side for enabling pressing on the reservoirs of the alkaline solution (thus dispensing the base on the paper supports) and two buttons that, once pressed, deliver the appropriate volume of solutions from the reservoirs to the paper supports (Figure 1B). The buttons can be raised for filling the reagent reservoirs and loading the EVOO sample using syringes. Finally, four windows on the lower side of the lab case allow imaging of the paper supports during analysis. It should be noted that, differently from the microfluidic system, the lab-case is reusable (it can be opened once the analysis has been completed to discard the microfluidic system).

The dark box (45 × 30 × 55 mm) connects the lab-case to the smartphone holder avoiding interference from ambient light. It also contains two additional components, a light diffuser for illumination of the paper supports using the integrated smartphone flash and a 0.4X two-lenses optical element to allow imaging of the paper supports by the smartphone camera.

The smartphone holder is specifically designed for the Samsung S8 smartphone (Samsung Group, Seoul, South Korea) to ensure correct positioning of the smartphone camera.

2.6. Analytical procedure for the quantification of total polyphenols in EVOO samples

The microfluidic system is enclosed in the lab-case and the reservoirs are filled with the reagents (alkaline solution, n-propanol, gallic acid standard solutions and blank) using syringes (this operation can be done in advance before *on-site* analysis). For the analysis, the sample loading chamber is filled by the EVOO sample with a syringe. Subsequently, the Na₂CO₃ solution is added to the four functionalized paper supports to activate the FC reagent by firmly pressing on the alkaline solution reservoirs in the central part of the lab-case. After 10 min, once the paper supports are dried, the two buttons of the lab-case are pressed simultaneously to deliver the EVOO sample (diluted 1:10

(v/v) with n-propanol), the two gallic acid standard solutions and the blank solution on the activated paper supports. Then, the dark box is connected to the lab-case and the smartphone is inserted in the holder. Five minutes upon delivering the solutions on the activated paper supports, the image of the paper supports is acquired with the dedicated app Camera FV-5 Lite, keeping the flash on during acquisition. Quantitative image analysis is performed using the freeware software ImageJ v.1.46 (National Institutes of Health, Bethesda, MD). For each image, regions of interest (ROIs) corresponding to the paper supports are defined and the colour intensity for each region is quantified as mean grey intensity (MGI). For each analysis, a calibration curve is obtained by plotting the MGI values of the two gallic acid standard solutions (previously normalized to the value measured for the blank) against the gallic acid concentration and fitting the data with a linear function. To obtain the total polyphenols content of the EVOO sample expressed in GAE, the MGI value of the sample is normalized as described above and interpolated on the calibration curve. The software GraphPad Prism v. 5.04 (GraphPad Software Inc., La Jolla, CA) is used to plot and analyse the experimental data.

3. Results and discussion

3.1. Optimization of paper-based FC assay procedure

The FC assay protocol was optimized for detection via smartphone imaging by both maximizing the blue-grey colour obtained in the presence of polyphenols and minimizing the background yellow shade due to unreacted FC reagent.

Preliminary experiments showed that the yellow colour of the FC reagent interferes with the quantitative analysis of polyphenols, causing an overestimation of the polyphenols content especially at low concentrations. To reduce the background colour, the amount of FC reagent deposited on the paper support was optimized by analysing 20 μL of a low-concentration ($10\text{ }\mu\text{g mL}^{-1}$) gallic acid standard solution in n-propanol. As shown in Figure 2A, the best result (i.e., a blue-grey colour with no detectable yellow shade) was obtained with paper supports functionalized with 5 μL of FC reagent.

Amount of base also represent a critical factor. In the standard FC assay [24] the base Na_2CO_3 is used to promote the chromogenic reaction with the FC reagent by converting the phenolic groups of polyphenols to phenolate anions. The optimal base amount to promote the FC reaction on paper

supports was determined by analysing 20 μL of a 75- $\mu\text{g mL}^{-1}$ gallic acid standard solution in n-propanol on paper supports activated with 15 μL of aqueous Na_2CO_3 solutions at different concentrations. Results reported in Figure 2B and 2C show that increasing Na_2CO_3 concentration up to 10% (w/v) yielded a gradual increase in developed colour intensity, while higher concentrations did not provide further improvement.

3.2. Calibration curve and assay performance

Total phenolic content of olive oil can vary depending on several parameters, such as region, variety, growing conditions, maturation, harvest time, and processing. Consequently, olive oil can display a total phenolic content ranging 50–1000 $\mu\text{g g}^{-1}$, the average content being in the range between 100–300 $\mu\text{g g}^{-1}$ [25]. To enable direct analysis of olive oil, the chemosensor was optimized in order to comprise this range of concentrations.

To perform direct analysis of polyphenols in EVOO without a preliminary extraction procedure, the EVOO sample have been diluted in a solvent that makes it compatible with the alcoholic environment in which the FC reaction takes place. n-Propanol, a low-cost and eco-sustainable solvent, was selected for this purpose. In order to simulate the EVOO matrix also in the solutions used to generate the calibration curve, gallic acid standards and blank were prepared in rice oil, which has a density similar to olive oil and a negligible polyphenol content (as measured with the standard FC assay).

To determine the optimal dilution of EVOO samples, we used the chemosensor to generate calibration curves by analysing gallic acid standard solutions prepared in rice oil diluted 1:3, 1:6 and 1:10 (v/v) in n-propanol and comparing the assay sensitivity (i.e., the slope of the calibration curve). As shown in Figure 3A, the 1:10 dilution (v/v) guaranteed the highest sensitivity, which can be reasonably attributed to a lower matrix effect (indeed, for the 1:3 dilution rice oil is not completely dissolved in the solvent). According to the calibration curve obtained in these experimental conditions (Figures 3B and 3C), polyphenols can be measured up to concentrations corresponding to 750 $\mu\text{g g}^{-1}$ of gallic acid equivalents in rice oil (considering the 1:10 (v/v) dilution factor of the sample). The detection limit (LOD) of the assay (calculated as the concentration of gallic acid giving a signal corresponding to that of the blank minus 3 times its standard deviation) was 30 $\mu\text{g g}^{-1}$ of gallic acid. This LOD value makes the method able to measure polyphenols even in EVOO samples with relatively low polyphenol content [23].

3.3. Chemosensor design

To perform total polyphenols quantitative analysis, the chemosensor has been designed to simultaneously analyze the EVOO sample (upon 1:10 (v/v) dilution in n-propanol) in parallel with two gallic acid standards (rice oil spiked with 250 or 500 $\mu\text{g g}^{-1}$ gallic acid and diluted 1:10 (v/v) in n-propanol) and the blank (rice oil diluted 1:10 (v/v) in n-propanol), which are employed to generate a three-point linear calibration curve during each assay. With this approach, the effect of any variation of experimental conditions is easily avoided, since the sample is analysed on a calibration curve simultaneously produced on the same cartridge. For example, when tested at temperatures in the range from 15 to 35°C, no significant variation on the results was observed.

The PDMS microfluidic system enclosed in the ABS lab-case thus contains four paper supports functionalized with the FC reagent and reservoirs preloaded with the reagents listed above. Upon loading the EVOO sample (20 μL) into the sample loading chamber with a syringe, it enables to perform activation of the FC reagent on paper supports by adding the base (10% (w/v) Na_2CO_3 solution), dilution of the EVOO sample with n-propanol and transfer of defined volumes of diluted sample, gallic acid standards and blank on the paper supports. Volumes of the reservoirs have been optimized considering the dead volumes of the fluidic channels, to deliver the proper amount of solutions (i.e., 20 μL) on the paper supports. To further simplify the analytical process and the design of the microfluidic elements, we investigated the possibility to use functionalized paper supports preactivated with the base. Unfortunately, such supports darken in a few hours, therefore activation of the paper supports with base must be performed just before analysis.

To obtain reliable quantitative results employing smartphone-based reflectometry measurements, a critical parameter is uniformity and reproducibility of illumination of the target area. For this purpose, the mini dark box was equipped with a PDMS light diffuser placed in front of the smartphone flash, and to improve light diffusion the surface of the light diffuser not in contact with the flash was covered with a reflective aluminium foil. To reduce the overall size of the dark box, it also contained a 0.4X wide-angle two-lenses optical element with a 140° viewing angle, which increases the image size by 1.4 times. This optical element allowed to reduce the focal distance of the smartphone camera by 70-80% (final focal length ~ 10 mm).

3.4. Assay validation and application to EVOO samples

The accuracy of the chemosensor regarding the sample dilution was evaluated on 1:10 (v/v) dilution of the oil sample with n-propanol. To this purpose, rice oil samples spiked with gallic acid were

analysed with the device and the results were compared with the actual gallic acid concentrations. As shown in Figure 4A, a good correlation between the measured concentrations and the real gallic acid amounts was observed, thus indicating the ability of the chemosensor to perform accurate and reproducible dilution of the sample.

A recovery study was carried out by analysing EVOO samples before and after spiking with gallic acid and comparing the increase in the measured total polyphenols with the amount of gallic acid added. As shown in Table 1, the recovery varies between 100 and 115%, thus confirming the specificity and accuracy of the chemosensor and the possibility to perform accurate total polyphenols analysis directly in the EVOO matrix.

Table 1. Recovery measured in EVOO samples spiked with gallic acid by using the smartphone paper-based chemosensor.

Gallic acid spiked ($\mu\text{g g}^{-1}$)	Measured total polyphenols ($\mu\text{g GAE g}^{-1}$) \pm SD ^a		Recovery (%) ^b
	Before spiking	After spiking	
100	310 \pm 8	423 \pm 13	113
200	310 \pm 8	540 \pm 7	115
300	310 \pm 8	611 \pm 13	100
400	310 \pm 8	709 \pm 19	100

^a Mean value of three independent measurements.

^b (Concentration of gallic acid measured after spiking - concentration of gallic acid measured before spiking) / (amount of gallic acid spiked).

Finally, to assess the accuracy of the chemosensor in the determination of total polyphenols in EVOO samples, the results obtained with the smartphone-integrated chemosensor were compared with those obtained on EVOO extracts using the standard FC assay performed in the 96-well microtiter plate format. The results obtained with the chemosensor (Figure 4B) were in good agreement with the total polyphenols measured with the standard FC assay, thus confirming the accuracy of the chemosensor. The assay also showed a satisfactory precision, providing a mean coefficient of variation (CV%) on real EVOO samples below 4%.

3.6. Stability of the chemosensor

To investigate the long-term stability of the chemosensor, we measured the changes of the analytical sensitivity (defined as the slope of the calibration curve generated during the analysis) during storage of PDMS microfluidic systems. Indeed, the paper supports functionalized with the FC

reagent because must be inserted into the PDMS microfluidic system when it is assembled, while the other reagents can be loaded just before insertion of the microfluidic system in the lab-case for performing the assay.

For the stability study, a series of PDMS microfluidic systems containing the paper supports functionalized with the FC reagent were stored in the dark at + 4°C, either under vacuum or in plastic bags. At regular time intervals, they were used to generate calibration curves and the slopes of the curves were compared. Figure 5, reporting the variation over time of the response of the chemosensor device, shows that the response obtained using the vacuum-packed PDMS microfluidic systems remains constant for at least 30 days, while that of elements stored in plastic bags showed a decrease of about 30%, presumably due to the effect of atmospheric oxygen. These results confirm the possibility to produce in advance PDMS microfluidic elements containing paper supports functionalized with the FC reagent and to store them until use without any significant decrease in sensitivity (paper supports functionalized for the analysis of aqueous samples showed a similar stability in time).

4. Conclusions

A portable, paper-based “all-in-one” chemosensor for the extraction-free determination of total polyphenols in EVOO exploiting the well-established colorimetric FC reaction and smartphone-based optical imaging detection was developed and validated. The chemosensor consisted in a disposable PDMS microfluidic system containing paper supports functionalized with the FC reagents and the other reagents required to perform the analysis and it also included ABS components (i.e., a reusable lab-case containing the microfluidic element, a mini dark box and a smartphone holder) for integration with a smartphone. Thanks to the design of the chemosensor, all the steps of the analysis can be performed in a simple and reproducible way by non-specialized personnel without reagent manipulation or use of pipettes or other volume measurement devices, requiring for the operator the simple task of adding the sample. In addition, the chemosensor is environmentally friendly, as it does not require extraction with solvents or use of toxic and polluting reagents.

Replica moulding and 3D printing techniques, which are commercially available at affordable prices, were used to realize all the components of the device. These technologies permitted rapid

prototyping, thus they made easy to implement changes in the design of the device and would also permit to adapt the device to other smartphones by simple producing different smartphone holders. It should be pointed out that, differently to the protocols and/or analytical devices already reported in the literature, this chemosensor allowed direct analysis of polyphenols in EVOO samples without any preliminary sample pre-treatment (e.g., sample extraction), thus greatly simplifying and shortening the overall analytical process (the assay time is about 15 minutes). The chemosensor is thus amenable for on-site application, which represents an important added value for its application in food and nutraceutical industry.

The chemosensor proved to be suitable for the accurate and reproducible quantification of total polyphenols in EVOO samples with a detection limit of $30 \mu\text{g GAE mL}^{-1}$. It was able to operate in a range of temperature from 15 to 35 °C without significant differences in the final results. In addition, any variation in the analytical performance as a function of experimental conditions is corrected by the internal calibration curve. The chemosensor could therefore be applied in the commercial enhancement of EVOO, allowing the rapid evaluation of one of its main quality parameters directly at the place of production or distribution. Moreover, we demonstrated that the microfluidic system containing the functionalized paper supports could be stored at +4°C in vacuum-sealed envelopes for up to 30 days without any loss of performance. When necessary, a stored microfluidic system can be rapidly prepared for use by filling the internal reservoirs with the reagents and enclosing it in the ABS lab-case. The same chemosensor can be readily employed for measuring polyphenols in other vegetal oil, simply by adjusting the concentration of gallic acid standard solutions with respect to the expected range of concentrations. This approach could be also exploited for performing other assays (e.g., antioxidant activity or acidity) upon transferring the assay in a paper format and developing - if not already available - a suitable optical reporting chemistry.

In conclusion, the combination of paper-based chemistry, simple microfluidics and smartphone-based optical detection allowed the development of a simple, easy-to-use portable analytical device for the on-site quantification of total polyphenols in EVOO. A possible further improvement could be to entrust the totality of the analysis to the smartphone by programming (taking advantage of the computing power of current smartphones) an integrated application for signal acquisition, quantitative image analysis and processing of the analytical data.

The direct analysis of other non-aqueous samples will be object of future additional application avoiding the time consuming pre-analytical steps of conventional assays.

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Figures

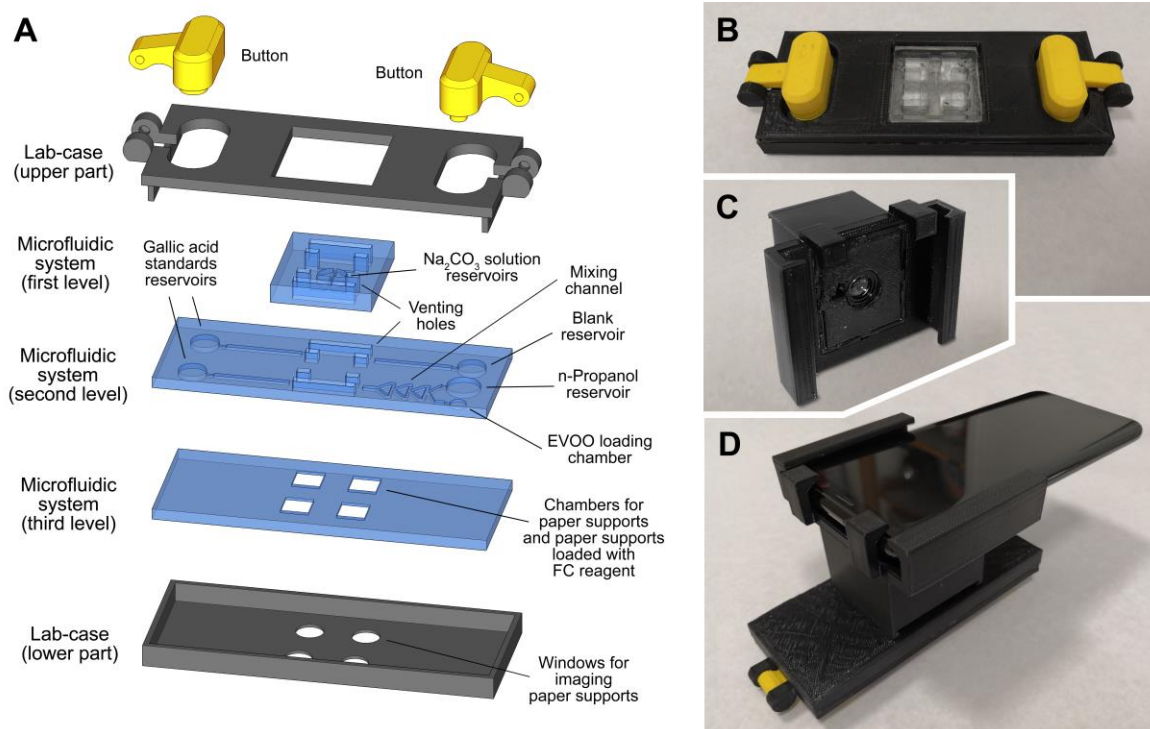


Fig. 1. Chemosensor for the measurement of total polyphenols in EVOO samples: (A) scheme of the PDMS microfluidic system and the lab-case, (B) assembled lab-case with the microfluidic system, (C) mini dark box, and (D) chemosensor with smartphone holder and smartphone.

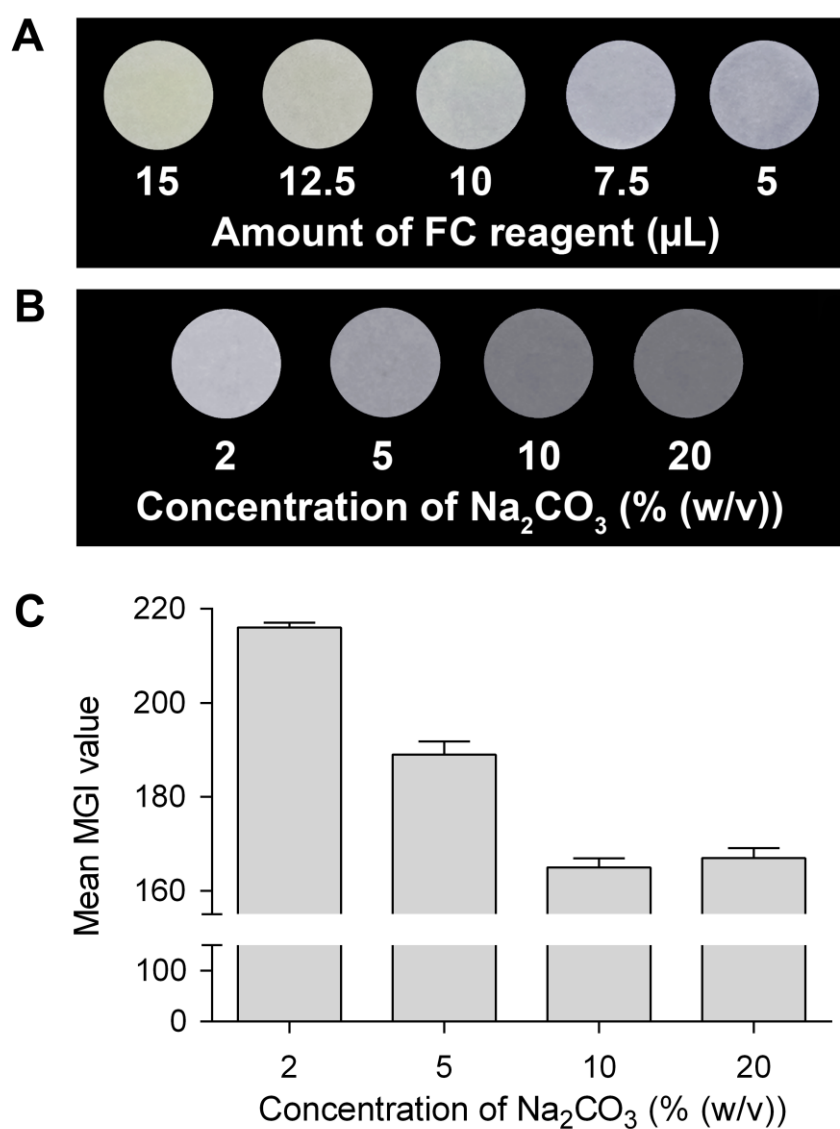


Fig. 2. (A) Images of paper supports functionalized with different amounts of FC reagent upon the analysis of a $10\text{-}\mu\text{g mL}^{-1}$ gallic acid standard solution. (B) Images of paper supports activated with aqueous Na_2CO_3 solutions at different concentrations upon the analysis of a $75\text{-}\mu\text{g mL}^{-1}$ gallic acid standard solution and (C) mean MGI values obtained with the different concentrations of base. Measurements were performed in triplicate.

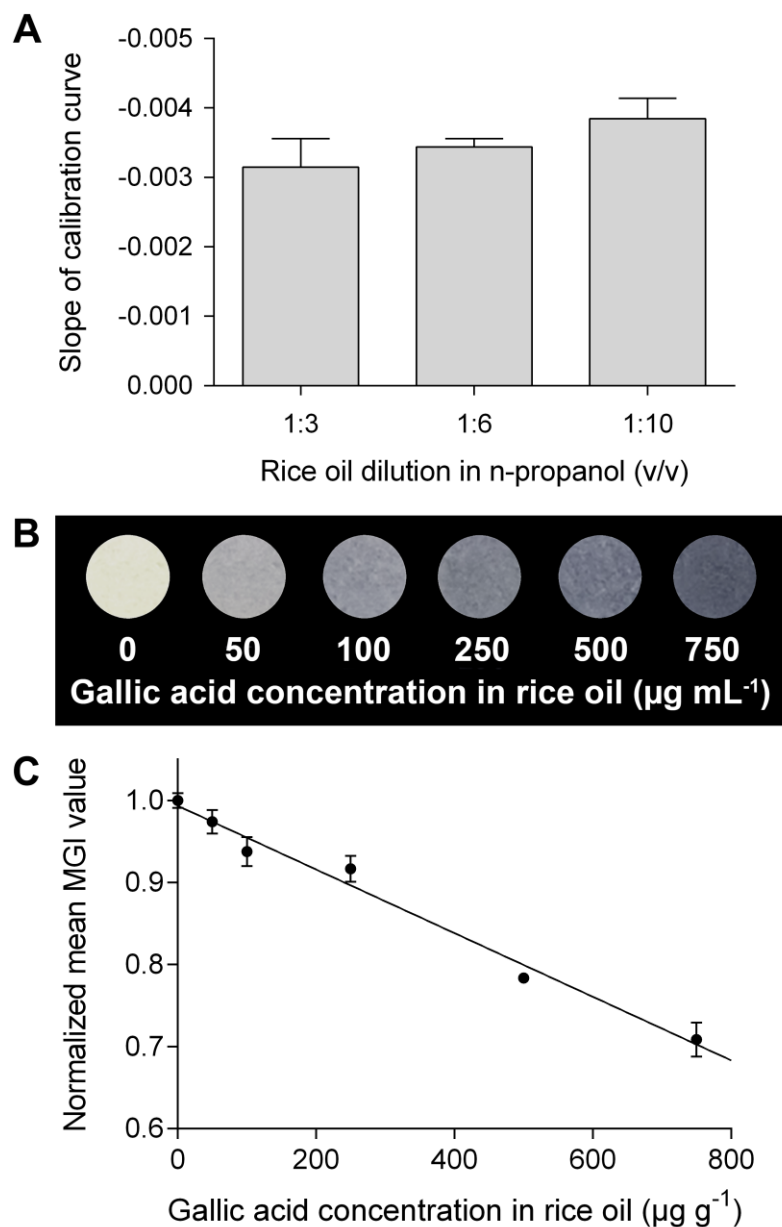


Fig. 3. (A) Slopes of the calibration curves obtained using gallic acid standard solutions prepared in different dilutions of rice oil diluted in n-propanol. (B) Images of paper supports and (C) calibration curve obtained in optimized experimental conditions for the analysis for the analysis of gallic acid standard solutions in rice oil, diluted 1:10 (v/v) in n-propanol. The equation of the calibration curve is $Y = (-0.000387 \pm 0.000024)X + (0.993 \pm 0.009)$ with $R^2 = 0.985$, where Y is the normalized mean MGI value and X is the gallic acid concentration ($\mu\text{g g}^{-1}$) of the rice oil solutions used for prepare the standards. Measurements were performed in triplicate.

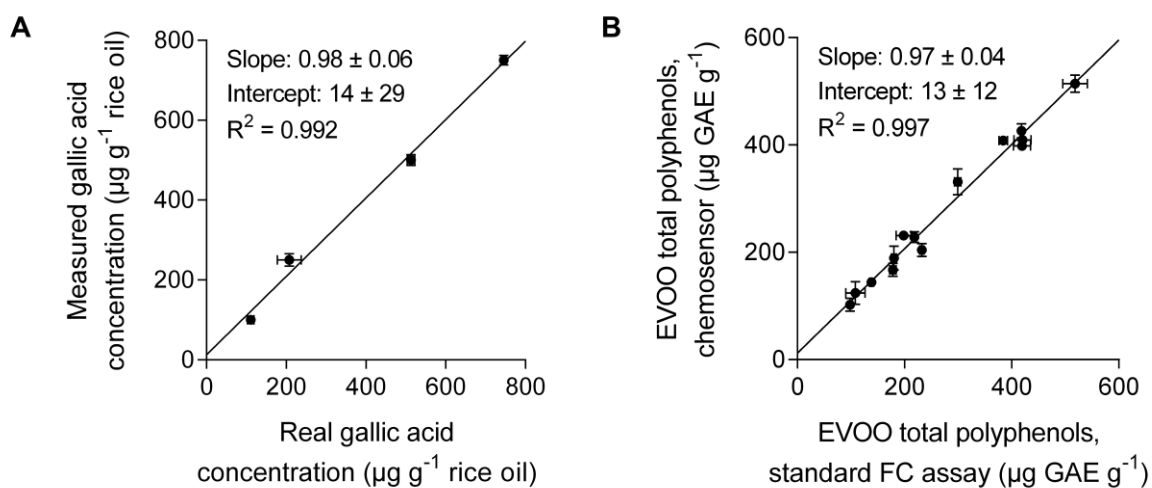


Fig. 4. (A) Comparison between gallic acid concentrations in rice oil samples measured with the chemosensor and real amounts of gallic acid. (B) Comparison between total polyphenols measured in EVOO samples with the chemosensor and with the standard FC assay. Measurements were performed in triplicate.

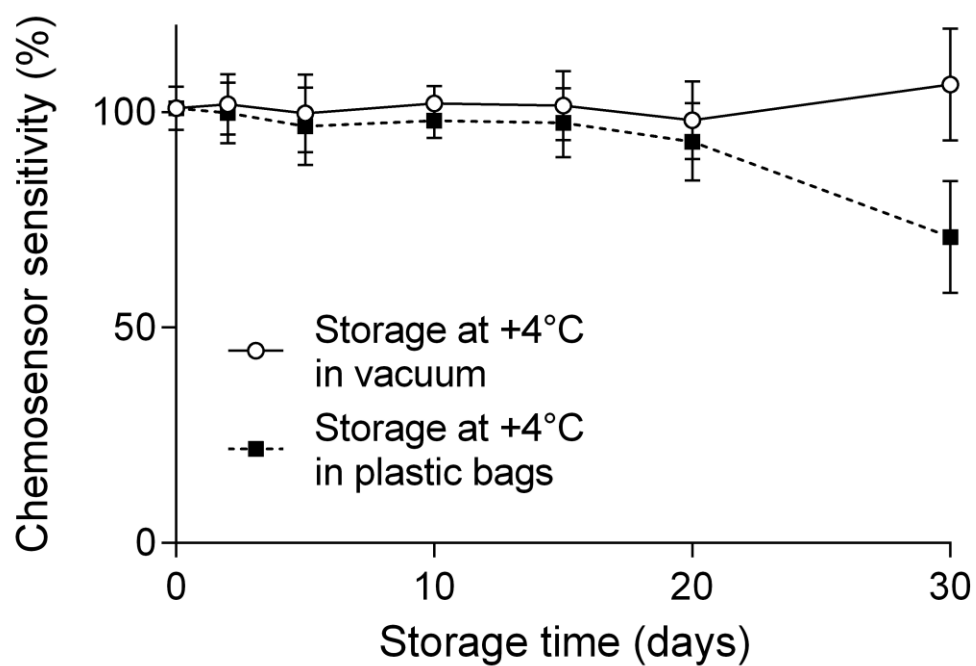


Fig. 5. Changes in the sensitivity of the chemosensor upon storage of the PDMS microfluidic element with functionalized paper supports in different conditions (the sensitivity obtained at time 0 was taken as a reference). Measurements were performed in triplicate.