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ARTICLE

Quercetin Determination in Dietary Supplement and in Onion Peel Extracts using a Photoluminescent 3-mercatpopropionic acid (3MPA) CdTe Quantum Dots Analytical Probe

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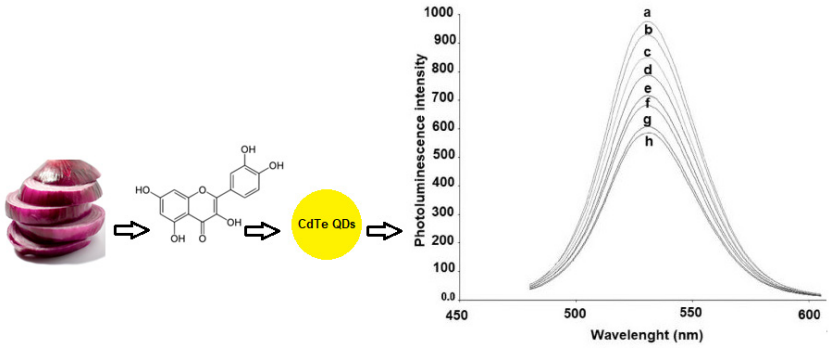

A method to quantify quercetin (QUE) was developed based on the quenching effect this flavonoid exerts on the photoluminescence of 3-mercaptopropioni acid (3MPA)CdTe quantum dots (QDs) in aqueous solution. The luminescence (460/527 nm) from 3MPA-CdTe QDs (at an estimated 1.5×10^{-7} mol L^{-1}) produced a linear relationship (R^2 0.990) between the luminescence quenched normalized signal in function of QUE concentration between 5.0×10^{-6} and 6.0×10^{-5} mol L^{-1}. The proposed method was successfully used to quantify QUE in the presence of other flavonoids and vitamin C with a limit of detection of 3.2×10^{-6} mol L^{-1}. The standard deviation at the 10^{-5} mol L^{-1} level of QUE was 2%. The effect of other flavonoids in QDs luminescence was evaluated and no interference was observed in the case of catechin and flavone (at concentrations as high as 5 times the one of QUE). Hisperitin, naringenin, kaempferol, and galangin showed no interferences at the same concentration of QUE. However, morin interfered when present even at the same concentration of QUE. Vitamin C interfered when present at a concentration higher than 10 times the one of QUE. The contents of QUE in manipulated formulation and food supplement capsules were determined by the proposed method and compared with results obtained by HPLC. Finally, quercetin determination with 3MPA-CdTe QDs was applied for the analysis of yellow and red onion extracts after thin-layer chromatography to enable selectivity towards QUE.

Keywords: quercetin, 3MPA-CdTe quantum dots, photoluminescence quenching, flavonoid, onion

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INTRODUCTION

Flavonoids are polyphenolic compounds occurring in plant-based foods, and they are known for their antioxidative,[1] antibacterial,[2] anticarcinogenic,[3] spasmolytic,[4] antiallergic,[5] and diuretic properties.[6] Flavonols are the most commonly found flavonoids in foods, with quercetin (QUE) and kaempferol as the main representatives[7] and important dietary constituents.[8,9]

Various methods have been proposed for QUE determination using HPLC,[10] voltammetry,[11] Raman spectroscopy,[12] tandem mass,[13] flow injection chemiluminescence (FI-CL) sensing using luminol-NaOH–H_2O_2 and molecularly imprinted polymeric microspheres,[14] capillary zone electrophoresis[15] UV-vis absorption spectrophotometry (direct measurement[16] or after complex formation[17]). Some of these techniques require expensive instrumentation, as well as complex sample preparation procedures.

Luminescent semiconductor quantum dots (QDs) present peculiar electronic and optical properties[18] due to their nanoscale size and quantum confinement effect. They are often constituted by elements from II-VI or III-V groups of the periodic table. In special, II–VI semiconductor nanocrystals as CdS, CdTe, CdSe, ZnS, HgTe have been extensively studied in many experimental and theoretical works.[19] They can also be obtained, in colloidal conditions, as structures comprising different semiconductors in a core-shell assembly. QDs present interesting characteristics in terms of luminescence such as a narrow and symmetric emission covering the near UV to infrared and high level of brightness and photostability.[20] Due to their high quantum yield, they are used at very low concentrations as probes. In addition, their photoluminescence emission can be adjusted by changing QDs size during synthesis.[21] These advantages have induced the development of sensors based on QDs.[22-26]

Through the literature, it is possible to observe an increasing tendency to relate QDs and flavonoids, exploring their interaction.[23,27-31,32] Jhonsi et al.[29] investigated the interaction of CdTe QDs, capped with thioglycolic acid, with some flavonoids developing a method to determine kaempferol by monitoring quenching of luminescence at 522 nm (limit of detection, LOD, of 0.79 μg mL^{-1}). CdTe QDs capped with 3-mercaptocarboxylic acid (3MPA) were used as sensitizers of chemiluminescence of the calcein/$K_3Fe(CN)_6$ system,[30] then developing a method to determine hyperin in seeds of *Cuscuta chinensis* Lam (LOD of 4.6 ng mL^{-1}) as it inhibit chemiluminescence induced by 3MPA-CdTe. The affinity of 7-hydroxyflavone and QUE with human serum albumin (HSA) was investigated, in the absence or presence of 3MPA-CdTe QDs of different sizes (diameters from 2.03 to 3.79 nm) and observed HSA spectral shifts and quenching effect were due to changes in the polarity of the immediate vicinity of protein tryptophan residues.[31]

Carvalho et al.[23] reported an analytical method using 2-mercaptopropionic acid capped CdS (2MPA-CdS) QDs for the determination of rutin, with a LOD of 1.2×10^{-6} mol L^{-1} (0.73 μg mL^{-1}).[23] Luminescence attenuation was caused by a combined inner filter effect and static luminescence quenching. Three phenolic compounds, hesperidin, hesperetin and QUE, were tested as interferents. The first two do not interfere even at concentration 10 times higher than rutin. In contrast, QUE promoted inner filter effect, therefore, it was separated from the sample containing rutin using thin-layer chromatography (TLC). The method enabled recoveries from 99% to 104% in saliva samples and in pharmaceutical formulations. Aucelio et al.[27] studied the interaction of several phenolic compounds with 3MPA-CdTe QDs in the presence and absence of a cationic surfactant (CTAB at 1.0×10^{-3} mol L^{-1}) aiming to obtain a relationship between the photoluminescence quenching, structure of the phenolic compound and its concentration.[27] In the absence of CTAB, morin, rutin, kaempferol and QUE produced quenching following a Stern-Volmer response, in which QUE interacted most strongly. In the presence of CTAB, rutin also produced quenching along with 3-hydroxyflavone and 7-hydroxyflavone.[27] Further studies evaluated the influence of the organized environment of 3MPA-CdTe QDs using CTAB aiming the interaction with five phenolic compounds, including QUE. It was shown that interactions are modulated by the inclusion of the phenolic compound into microenvironments of surfactant aggregates around QDs. Changing in photoluminescence of QDs was related to chemical structure of the phenolic compounds, and to the surrounding characteristics of the dispersed QDs.[31] Karuppusamy et al.[32] developed an electrochemical sensor comprising of CdTe QDs boron- sulphur co-doped reduced graphene oxide to determine QUE to analyze apple juice samples (LOD of 0.018 μmol L^{-1}).

In this work, 3MPA-CdTe QDs were used as luminescent probe for the determination of quercetin in samples containing other flavonoids and vitamin C in aqueous medium. The method was simple and convenient for QUE determination in food supplement, manipulated formulation, as well as in yellow and red onion extracts.

MATERIALS AND METHODS

Apparatus

Absorption spectra were made on a Lambda 35, UV/Vis/NIR double beam spectrometer (Perkin Elmer, UK) using 10 nm spectral band pass, 240 nm min^{-1} scanning rate using quartz cuvette with 1 cm optical path length. Photoluminescence measurements were made in a LS 45 luminescence spectrophotometer (Perkin-Elmer) using 10 nm excitation and emission spectral band pass and 1200 nm min^{-1} scanning rate with solutions placed in 1 cm optical path length quartz cuvette. Chromatographic analyses were made using a high-performance liquid chromatographic system (Agilent Technologies, 1200 series) with a diode array detector (absorbance) and Zorbax Eclipse XDB-C18 column (4.6 x 250 mm, 5 mm particle size). The ultrasonic bath used for degassing solvents was the USC 1800 model (Unique, Brazil).

Reagents

QUE, rutin hydrate (RUT), naringenin (NAR), morin (MOR), kaempferol (KAEMP), hisperitin (HSPT), flavone (FLAV), galangenin (GAL), catechin (CAT), cadmium acetate dehydrated, $CdCl_2 \cdot 2.5H_2O$ (98%), tellurium powder (200 mesh), 3-mercaptopropionic acid (3MPA), sodium borohydride and ascorbic acid were acquired from Sigma Aldrich (USA). Methanol, acetic acid, hydrochloric acid and sodium hydroxide were of analytical spectral grade (Merck, Germany). The TLC silica gel plates were also obtained from Merck. Ultrapure water was collected from an ultra-purifier Gradient A10 Milli-Q system (Millipore, USA). The commercial pharmaceutical Quercetin – Food Supplement plus Vitamin C (Supplement Spot) containing 500 mg QUE / 200 mg vitamin C (in two capsules) was purchased in a local drugstore and the 200 mg QUE / 200 mg RUT formulation was made in a local pharmacy. Red and yellow onions were purchased in a local market (Rio de Janeiro, Brazil). Nitrogen gas was obtained from Messer (Brazil).

3MPA-CdTe QDs synthesis

Both, NaHTe solution and 3MPA-CdTe QDs were prepared as indicated in literature.[33] Amounts of $CdCl_2$ (0.20 mmol) and 3MPA (0.4 mmol) were mixed in water before pH adjustment (pH 10.0) using small aliquots of additions of a NaOH solution (1.0 mol L^{-1}). The solution was transferred to a three-necked flask and purged with nitrogen before the addition of a fresh solution of NaHTe (2.0 mL) under stirring at room temperature. In these conditions, Cd:3MPA:Te proportion was 1:2:0.2. The reaction was kept under reflux (at 100 ºC) under inert atmosphere until proper emission maximum wavelength (related to 2.7 nm diameter nanocrystal) was achieved.

The characterization of 3MPA-CdTe QDs was made as described by in a previous work[27] with average diameter of 2.7 nm and quantum yield about 50%, adjusting concentration of the stock dispersion of about 1.5×10^{-7} mol L^{-1} and excitation and emission pair ($\lambda_{ex/em}$) of 460/527 nm.

Preparation of standard solutions and samples

Standard stock solutions (at 1.0×10^{-2} mol L^{-1}) were prepared by dissolving specific amounts of flavonoids in methanol. Phosphate buffer (0.01 mol L^{-1}) was prepared using monosodium phosphate (0.01 mol L^{-1}) and its conjugate base, disodium phosphate (0.01 mol L^{-1}) to adjust the pH. The working 3MPA-CdTe aqueous dispersions were prepared in volumetric flasks (10.00 mL) by adding 1.00 mL of phosphate buffer (0.01 mol L^{-1} at pH 7.4), 60 µL of aqueous dispersion of 3MPA-CdTe (at 1.5×10^{-7} mol L^{-1}), and appropriate volumes of the stock solution of QUE (1.0×10^{-2} mol L^{-1} prepared in methanol). Final methanol volume adjusted was made to achieve 1.00 mL (10% in volume) before final adjustment of dispersion using ultrapure water (10.00 mL). These dispersions were left resting for at least 45 min before

performing measurements allowing the interaction between QUE and 3MPA-CdTe reach equilibrium. Analytical curve was prepared in the same way. In order to study interferences, 5.0 µL of QUE solution (1.0×10^{-2} mol L^{-1}) and 5 or 25 µL of a 1.0×10^{-2} mol L^{-1} solution containing a potential interferent were added in 10.00 mL volumetric flasks. Finally, methanol was added completing 1.00 mL (10% in volume) before adding ultrapure water to obtain 10.00 mL final volume with QUE at 5.0×10^{-6} mol L^{-1} and with potential interferents at 5.0×10^{-6} mol L^{-1} or at 5.0×10^{-5} mol L^{-1}.

Ten capsules of Dietary Supplement Quercetin containing QUE/Vitamin C (500 mg / 250 mg in two capsules) or ten capsules of manipulated formulation QUE/RUT (200 mg / 200 mg per capsule) were weighed and pulverized. An appropriate amount of the powder (aiming to achieve a theoretical 1.0×10^{-2} mol L^{-1} of QUE) was mixed with methanol. After vigorous agitation, the mixture was filtered on a PTFE syringe filter (0.45 µm) and collected in a 10.00 mL volumetric flask with final volume adjusted using methanol. Sample were prepared by adding an amount of supplement stock solution, 1.00 mL of phosphate buffer (0.01 mol L^{-1} at pH 7.4), 1.00 of mL methanol, and 60 µL working 3MPA-CdTe QDs dispersion, and the final volume was achieved by addition ultrapure water. Measurements were made after 45 min of preparation allowing the interaction between QUE and 3MPA-CdTe reach equilibrium. For the manipulated formulation sample (aiming 1.0×10^{-2} mol L^{-1} of QUE) was prepared in the same manner.

The extracts of onion peels were obtained according to the method proposed by Hertog et al.[34] with minor modifications. Onion peels were washed with ultrapure water, then resting at room temperature for 24 h to dry in open air. Subsequently, 10.0 g of each type of onion (red and yellow) peel were weighed and cut into small pieces and placed in a round-bottom flask containing 80 mL methanol and 20 mL of HCl solution (1.2 mol L^{-1}). The mixture remained under reflux (90 ºC) for 2 h. The extracts were transferred to beakers, filtered and concentrated on a hot plate at approximately 60 ºC. Finally, the final volume of each extract was completed to 10.00 mL by adding appropriate amounts of methanol. For each type of onion, three independent extracts were obtained.

HPLC analyzes

Samples of manipulated formulation containing QUE and RUT and a food supplement containing QUE and Vitamin C were also analyzed by HPLC. Volumes of 20 e 40 µL of a 1.0×10^{-2} mol L^{-1} (expected concentration of QUE) of a manipulated formulation solution and 10 and 20 µL of a 1.0×10^{-2} mol L^{-1} (expected concentration of QUE) of a food supplement solution were placed in 10.00 mL volumetric flasks with final volume adjusted using methanol. Sample solutions were prepared by dissolving them directly with methanol and filtering before further dilution. Detection of absorbance was made at 254 nm. The mobile phase was constituted by aqueous solution 0.05% trifluoracetic acid (% v/v) (solvent A) and acetonitrile (solvent B) at 1.0 mL min^{-1} flow rate. The chromatographic method[35] for the determination of QUE in solutions containing QUE/RUT and QUE/vitamin C used gradient regime with 5% acetonitrile in time zero, increasing to 100% after 15 min. In such conditions, the QUE retention time was 10.0 min. The analytical curve of QUE was constructed by the introduction (15 µL) of different concentrations of standard solution of QUE (1.0; 2.0; 3.0; 4.0 and 6.0×10^{-5} mol L^{-1}) Sample solutions aliquots of 15 µL were introduced for analysis.

Thin-layer chromatography (TLC)

QUE analytical curves on TLC were obtained by spotting different volumes of QUE standard solution 2.0×10^{-2} mol L^{-1} (in methanol) onto TLC plates (without fluorescence indicator) with dimensions of 5.0×8.0 cm. After methanol dried, the plates were placed into beakers that were covered with watch glasses to prevent evaporation of the mobile phase constituted by hexane/ethyl acetate/acetic acid 20/19/1, v/v/v. QUE spot was isolated (RF of 0.35) removed from the plates together with the silica gel and the resulting solid (about 0.05 g) was placed in 10 mL beakers. The identification of the characteristic RF was made in a previous study by staining the chromatographic plate with iodine vapor.[23] Then, 3.0 mL aliquots of methanol were added to each beaker, and the mixtures were taken to an ultrasonic bath for 5 min.

The mixtures were then filtered using syringes and PTFE filters (0.45 μm) and collected in 10.00 mL volumetric flasks. The solvent was evaporated under a flow of N_2 and the solid residue inside the volumetric flask was re-dissolved following the composition of the 3MPA-CdTe QDs probe. For onion extracts, the same procedure was followed by spotting 15 μL of extract on the TLC.

RESULTS AND DISCUSSION

Experimental conditions for photoluminescence

Experimental conditions were studied in order to achieve stable photoluminescence from the 3MPA-QDs in aqueous dispersions. The volume of the 3MPA-QDs stock dispersions added to the working dispersion was 60 μL (1.5×10^{-7} mol L^{-1} estimated final concentration of 3MPA-CdTe QDs) and final pH of the aqueous dispersions was adjusted (by adding HCl or NaOH solution aliquots). The pH choice was made as it was verified that dispersions with pH in the acidic range presented a significant decreasing in photoluminescence. Photoluminescence at pH 7.0, 7.4 and 8.0 showed equivalent photoluminescence intensity with fairly stability over at least 60 min. In contrast, photoluminescence intensity decreased by around 30% in systems adjusted at pH 9.0. As pH 7.4 was chosen, phosphate buffer was tested to adjust pH at three concentrations (1.0 mL of 0.01, 0.02 or 0.04 mol L^{-1} buffer solution in a final volume of 10.00 mL) and it was observed the decreasing in signal as the phosphate buffer concentration increased. Therefore, 1.0 mL of the 0.01 mol L^{-1} phosphate buffer at pH 7.4 was used.

In order to guarantee complete dissolution of the QUE, different volumes of methanol (0.5, 1, 2, 3 and 4 mL) were introduced in the composition of the 10.00 mL aqueous working dispersions of 3MPA-CdTe QDs. The inclusion of 0.5 or 1.0 mL methanol promoted the increasing of signal intensity (about 25% higher) probably due to the solubilization of QUE. However, volumes higher than 1.0 mL of methanol decreased the signal intensity considerably (down to about 60% with 2.0 mL of methanol). The addition of 1.0 mL of methanol did not affect stability of QDs in the dispersion (including intensity over at least 60 min), therefore this volume was chosen to comprise the composition of the working dispersion.

Photoluminescence quenching of CdTe-3MPA QDs induced by QUE and method validation

It has been proven, in a previous work concerning interaction mechanism between flavonoids and CdTe QDs, that quenching promoted by QUE is of static nature as the increasing of the temperature (from 20 ºC to 32 ºC) promoted the decreasing in efficiency of the quenching effect as the QUE/3MPA-CdTe QDs complex is disrupted favoring QDs excitonic recombination.[27]

QUE presents a wide absorption profile between 200 and 400 nm (Figure 1) promoting potential inner filter effect as photoluminescence of QDs overlaps this same range. Therefore, the QUE absorption spectra must be considered in choosing the excitation wavelength for 3MPA-CdTe QDs. The maximum absorbance of QUE (at 5.0×10^{-5} mol L^{-1}) was 1.08 at 385 nm, consequently the excitation of QDs was chosen to be at 460 nm (out of the strong influence of the absorption of QUE and within the first excitonic profile of 3MPA-CdTe QDs). Under this condition, it is possible to minimize influence of the absorption of QUE still allowing measurement of the relatively intense photoluminescence from QDs at 527 nm. At 460 nm, the absorbance of QUE (A_{460} at 6.0×10^{-5} mol L^{-1}) was 0.046 while at 527 nm (where is the emission maximum of the 3MPA-CdTe QDs), absorbance is 0.0084.

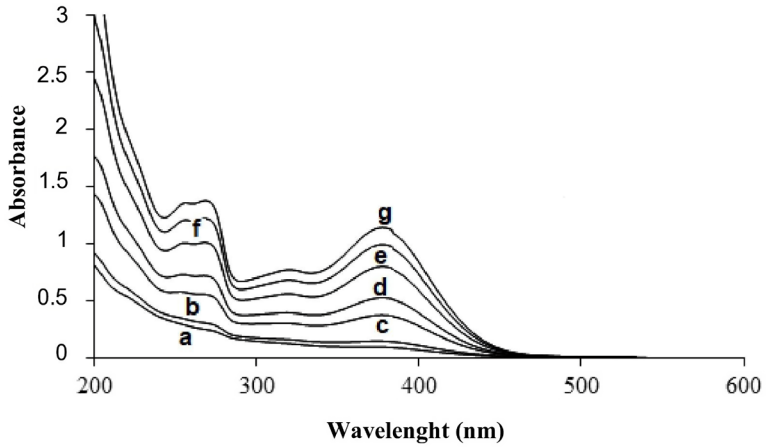

Figure 1. Absorption spectrum of 3MPA-CdTe QDs dispersions (1.54×10^{-7} mol L^{-1}, line a) in the presence of QUE: (a) 0.5, (b) 1.0, (c) 2.0, (d) 3.0, (e) 4.0, (f) 5.0 and (g) 6.0×10^{-5} mol L^{-1}.

Despite the fact that excitation/emission was chosen to keep inner filter effect to a minimum, it is still necessary to evaluate the calibration model in order to rule out any possibility of systematic error. The potential inner filter effect, caused by absorption at different concentrations of QUE (5.0×10^{-6} to 6.0×10^{-5} mol L^{-1}), in the photoluminescence (measured at $\lambda_{ex/em}$ of 460/527 nm) was corrected (L_{corr}) using Equation 1. Then, the normalized model using the ratio of luminescence in absence (L_0) and in the presence (L) of QUE was constructed. In this model, the L_0/L ratio in function of the concentration of QUE, [*QUE*], produced a linear response with the sensibility (angular coefficient) equivalent to the static quenching constant K_S as seen in Equation 2. The K_S calculated through this inner filter corrected data was 1.09×10^4 L mol^{-1} which was slightly higher than the one obtained under inner filter effect (1.01×10^4 L mol^{-1}). A sequence of photoluminescence spectra from the 3MPA-CdTe QDs probe is shown in Figure 2 as the concentration of QUE was increased from 0.5 to 6.0×10^{-5} mol L^{-1}.

$$L_{corr} = L \times 10^{[(A_{ex} + A_{em})/2]} \qquad \text{Equation 1}$$

$$L_0/L_{corr} = 1 + K_S\,[QUE] \qquad \text{Equation 2}$$

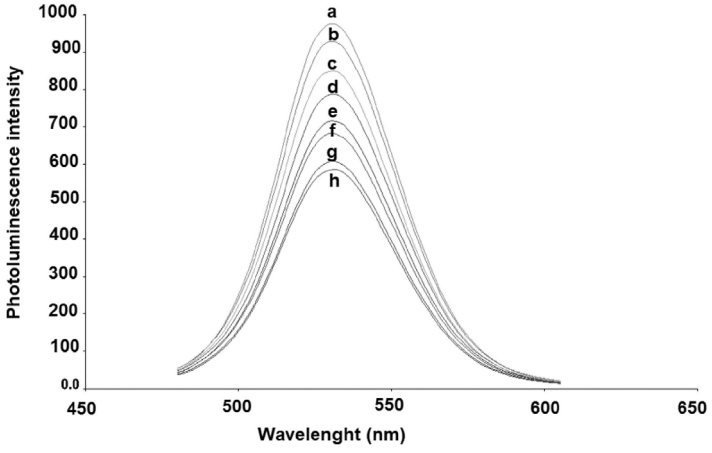

Figure 2. Photoluminescence of 3MPA-CdTeQDs with quenching proportional to the increase QUE concentration: (a) 0, (b) 0.5, (c) 1.0, (d) 2.0, (e) 3.0, (f) 4.0, (g) 5.0 and (h) 6.0×10^{-5} mol L^{-1} of QUE.

Luminescence normalized plots in function of the concentration of QUE (analytical curves) are shown in Figure 3, with analytical curve equation of $L_0/L = 1.01 \times 10^4$ *[QUE]* + 1.01 (R^2 = 0,9907) without inner filter effect correction and $L_0/L = 1.09 \times 10^4$ *[QUE]* + 1.01 (R^2 = 0,9938) for the one corrected inner filter effect indicating a difference in sensibility of about 7%. The results showed that absorption contribution of QUE at both 460 nm and 527 nm is very small, thus choosing appropriate excitation/emission pair is important to minimize interference when using the 3MPA-CdTe QDs probe to quantify QUE. Linear range covers two orders of magnitude, which it is inherent for luminescence quenching based methods. This imposes previous evaluation of sample to adjust dilution in function of signal variation as it is also common for methods based on UV absorbance measurement including HPLC-UV.

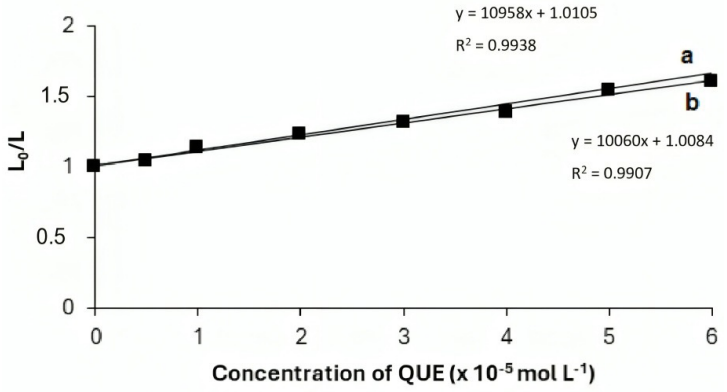

Figure 3. Normalized photoluminescence quenching from 3MA-CdTe QDs probe by QUE: 0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 $\times$ 10^{-5} mol L^{-1}, (a) uncorrected curve and (b) inner filter effect corrected curve. Standard deviations of replicates were less than 1%.

The detection limit of 1.3×10^{-6} mol L^{-1} was calculated based on the concentration of QUE capable of reduce average photoluminescence of the dispersion of QDs (x_b), to a value equal to $x_b - 3s_b$, where s_b represents the standard deviation of 10 measurements of the blank (working dispersions without QUE). The quantification limit of 4.8×10^{-6} mol L^{-1} was calculated as the concentration of QUE capable of reducing the mean probe signal (x_b) to a value equal to $x_b - 10s_b$. To assess the precision of the method, it was used the propagated standard deviation (Equation 3). Measurements were carried out and the variation of the L_0/L value for a set of 10 independents solutions was obtained for QUE at two concentration levels. The $S_{(L0/L)}$ of 2.9 and 2.5 % were found for 2.0×10^{-5} and 4.0×10^{-5} mol L^{-1} respectively.

$$S_{(L0/L)} = L_0/L \times [(S_1/L)^2 + (S_{l0}/L)^2]^{1/2} \qquad \text{Equation 3}$$

Intermediate precision was evaluated and expressed as coefficient of variation (CV) based on ten independent dispersions QUE (at 2.0×10^{-5} mol L^{-1} and 4.0×10^{-5} mol L^{-1}) with signal measured at 527 nm interpolated onto the analytical curve. Two days later, new analyses were performed at the same concentration levels. On the first day, the average concentration of QUE found was 2.02×10^{-5} mol L^{-1} with CV of 0.106%. On the second day, an average QUE concentration of 2.00 x 10^{-5} mol L^{-1} was found with CV of 0.086%). The similarity of variances was proven using the F test ($s^2_{largest}/s^2_{smallest}$) ($n_1 = n_2 = 10$, degrees of freedom of the numerator and denominator = 9), where the F_{calc} value was 2.7 (with F_{tab} of 3.18). For the concentration of 4.00×10^{-5} mol L^{-1}, the results of the tests performed on the first day indicated an average concentration of 4.10×10^{-5} mol L^{-1} with CV of 0.041%.The average concentration found on the second day was 3.9×10^{-5} mol L^{-1} with CV of 0.09%. Since the F_{tab} value is greater than that of F_{calc}, it can be concluded that variances of measurements performed on two consecutive days are similar and the proposed method has satisfactory intermediate precision for the two concentration levels tested.

The variation of the analytical curve was also verified using the standard deviation of the K_S obtained from curves constructed in three different days. The K_S values were 1.10×10^4, 1.04×10^4 and 1.12×10^4 L mol^{-1}. The mean obtained was 1.08×10^4 L mol^{-1}, with a standard deviation of 0.05 L mol^{-1} with coefficient of variation of 4.3%.

Accuracy of the method was evaluated by means of recovery assays, in ten independent 3MPA-CdTe QDs dispersions containing QUE at two concentration levels. The average of the photoluminescence (ten sample measurements) was interpolated on the analytical curve and results compared with the values of the expected concentrations. The percent recoveries were 100 ± 1.7% and 103 ± 1.5% for the concentrations of 2.0×10^{-5} and 4.0×10^{-5} mol L^{-1} of QUE, respectively.

Interferences from other phenolic compounds and vitamin C

Many compounds have the potential to disturb QDs photoluminescence, therefore, in order to check the selectivity of the response towards QUE, the presence of other phenolic compounds (flavona, naringenin, morin, catechin, hispertin, rutin, kaempferol and galangin) was tested in order to evaluate their influence on 3MPA-CdTe QDs photoluminescence. The study compared the photoluminescence measured from the probe in the presence of QUE (L_{QUE}) with the ones measured in the presence of QUE together with another phenolic compound ($L_{(QUE+phenolic\ compound)}$) at the QUE:phenolic compound molar proportions adjusted to either 1:1 or 1:5 with QUE set at 5.0×10^{-6} mol L^{-1}. Results reported in percent recovery, ($L_{(QUE+phenolic\ compound)}$)/$L_{QUE}$) × 100, are placed in Table I and no significant interferences were found from catechin and flavone (at proportions as high as 5 times the one of QUE). Hisperitin, naringenin, kaempferol, morin and galangin did not interfere when present at the 1:1 proportion with QUE. However, interference was found at the 1:5 proportion. Absorption of rutin is similar to the one of QUE which causes significant inner filter interference. A detailed study with QUE/rutin from 1:1 to 1:10 molar proportion was made showing the degree of interference (Table II). For the interferences study, recovery below to 90 % was defined as interference.

Table I. Recoveries of QUE (5.0×10^{-6} mol L^{-1}) in samples solution QUE fortified with other organic compounds in a molar proportion 1:1 and 1:5

Interference	Recovery (%) 1:1 QUE/organic compound	Recovery (%) 1:5 QUE/organic compound
Morin	91.6	80.4
Hisperitin	99.6	82.1
Catechin	103	99,4
Naringenin	96.8	84
Flavone	97.9	92.2
kaempferol	92.2	77.1
Galangin	100.1	81.8
Rutin	98.7	87.1
Ascorbic acid	100.8	106.5

Since QUE and vitamin C can often be associated in pharmaceutical preparations, the selectivity in photoluminescence response of 3MPA CdTe QDs probe towards QUE was evaluated in presence of vitamin C at a molar concentration 10 times higher than the one of QUE. It was found that vitamin C promoted an increase in signal variation of about 60% higher than the one observes only with QUE.

Table II. Recoveries of QUE in the presence of rutin from different molar proportions (1/1 to 1/10 QUE/rutin)

Molar ratio	Recovery (%)
1/1	97.8
1/2	95.2
1/3	91.8
1/4	90.3
1/5	87.1
1/6	85.4
1/7	84.9
1/8	82.9
1/10	81.6

Separation and isolation of quercetin by thin-layer chromatography

The separation of QUE is required considering the complexity of the onion extract sample matrices. Therefore, a simple TLC procedure was proposed despite the fact that including this step increases analysis time and complexity in operation, also limiting potential for fully automation of the method. Chromatography conditions were adjusted using QUE standards, and then the procedure was applied to the separation of QUE present in onion extract samples, isolating QUE spot at a RF 0.35 (visually identified by a yellowish color stain). Evaluation of a possible interference imposed by the silica gel matrix on the photoluminescent probe was carried out as described in the Materials and Methods section. The results indicated an attenuation in the intensity of the photoluminescent signal of 6 ± 1% (n = 6). Therefore, all QUE standards were also run through the TLC plate prior to the construction of the analytical curve.

The evaluation of the analytical curve response was made by preparing QUE standards collected from TLC was performed on different days (each day an analytical curve was constructed, totaling five days). The curves were prepared by spotting different volumes of QUE standard solution 2.0×10^{-2} mol L^{-1} (5, 10, 15 and 20 µL) on the TLC plate so as to have added masses of 30, 60, 91 and 120 µg, respectively. The analytical curves showed linear ranges between 1.0 and 4.0×10^{-5} mol L^{-1} (or between 30 to 120 µg in absolute mass values) with equations indicated in Table III. The difference in the average sensitivities between the curve corrected and uncorrected for the filter effect was 4.1%. Considering the small difference in the parameters of the corrected and uncorrected curves and the inherent standard deviation of K_s (as seen in previous sections) analysis was performed using L_0/L uncorrected ratios. In terms of selectivity there is always possibility of co-elution of other polyphenols but these are expected to be in much lower content compared to QUE in the analyzed samples. Besides, it is also known that many of them do not interact with CdTe QDs to produce significant disturbance in photoluminescence.[27]

Table III. Parameters of the normalized luminescence (L_0/L) normalized curve equations of QUE obtained on TLC with and without inner filter effect correction

Day	Corrected curve equation	R^2	Uncorrected curve equation	R^2
1	$y = 8100x + 1.002$	0.998	$y = 8500x + 1.003$	0.996
2	$y = 7700x + 1.004$	0.993	$y = 7950x + 1.006$	0.993
3	$y = 8600x + 0.992$	0.992	$y = 8990x + 0.997$	0.993

(continuation on next page)

Table III. Parameters of the normalized luminescence (L_0/L) normalized curve equations of QUE obtained on TLC with and without inner filter effect correction (continuation)

Day	Corrected curve equation	R^2	Uncorrected curve equation	R^2
4	$y = 7600x + 1.002$	0.997	$y = 7900x + 1.003$	0.995
5	$y = 7800x + 1.008$	0.995	$y = 8100x + 1.007$	0.995

Samples analysis

The method was tested to determine QUE in food supplement containing 500 mg of QUE and 200 mg vitamin C (content of two capsules), also in manipulated formulation containing rutin. Sample stock solutions were prepared using appropriate sample quantities leading to an expected QUE concentration of 1.0×10^{-2} mol L^{-1}. From this, sample solutions at two QUE concentration levels (2.0×10^{-5} and 4.0×10^{-5} mol L^{-1} for manipulated formulation and 1.0 and 2.0×10^{-5} mol L^{-1} for food supplement) were prepared. Analyses made using the 3MPA-CdTe QDs probe were compared with results obtained HPLC. Results obtained by the proposed method and the HPLC method (reference) are displayed in Table IV and agree with recoveries using the proposed method satisfactory (n = 3).

For onions (yellow and red onions), each extract was used to prepare three independent solutions, and these were spotted on the chromatoplates, following the conditions described in materials and methods section. The red onion extracts showed an average QUE concentration of $(3.6 \pm 0.4) \times 10^{-5}$ mol L^{-1} (equivalent to 10.7 ± 1,1 mg per 10 g of dry peel). In the yellow onion extracts, the average concentration was $(2.7 \pm 0,2) \times 10^{-5}$ mol L^{-1} (equivalent to 8.1 ± 0,3 mg per 10 g of onion peel. Standard concerns the repeatability of the analysis.

Table IV. Analysis of food supplement and manipulated formulation by the proposed method and reference procedure

Sample	Expected value (mol L^{-1})	Recovered value (mol L^{-1})		Recorery[a] (%)
		HPLC	Proposed method	
Manipulated formulation	2.0×10^{-5}	$1.9 \pm 0.16 \times 10^{-5}$	$2.02 \pm 0.18 \times 10^{-5}$	106.9
	4.0×10^{-5}	$3.9 \pm 0.2 \times 10^{-5}$	$4.03 \pm 0.25 \times 10^{-5}$	103.3
Food supplement	1.0×10^{-5}	$1.06 \pm 0.6 \times 10^{-5}$	$1.03 \pm 0.07 \times 10^{-5}$	97.2
	2.0×10^{-5}	$2.02 \pm 0.10 \times 10^{-5}$	$1.98 \pm 0.14 \times 10^{-5}$	98.0

[a](Proposed method recovery/HPLC recovery) × 100

The analyses of the onion extracts were also performed with interpolation on the analytical curves constructed passing through standards in TLC plates. Two analytical curves were made (one on each day), and the determinations of QUE in the extracts indicated that the results agreed with the previous ones, as seen in Table V. The test proved that there was no statistical difference in the concentrations of QUE, which demonstrates that the construction of TLC curves is not mandatory. However, the use of TLC is necessary for the separation and isolation of quercetin from the other constituents of the extracts due to the complexity of the matrix of the onion samples.

The Student's *t*-test performed for analyses carried out in two different days indicated that there was no significant difference among results obtained in the determinations (t_{Exp} of 0.93 and 0.44 for red and yellow onions, respectively, which are lower than the test`s critical value (t_{tab}=2.78).

Table V. Determinations of QUE in extract onion samples

Sample	Concentration ($\times 10^{-5}$ mol L^{-1})	Concentration (mg / 10 g)	Concentration ($\times 10^{-5}$ mol L^{-1})	Concentration (mg / 10 g)
	Day 1	Day 1	Day 2	Day 2
Red onion	3.3 ± 0.7	9.9 ± 2.0	3.8 ± 0.7	11.5 ± 2.0
Yellow onion	2.6 ± 0.5	7.9 ± 1.7	2.7 ± 0.6	8.3 ± 1.7

Greenness evaluation

The AGREE calculator[36] was used to evaluate environmental impact the analytical method. This procedure generated the segmented circular chart (with colors varying from red to dark green) reflecting the performance of the 12 specific aspects (taking into consideration principles of Green Analytical Chemistry) and also of the method as a whole (Figure 4), which obtained the overall 0.58 out of the ideal score (1.0).

The result indicated green/yellow in half of the evaluated aspects. Falls in the greenish range of colors (high scores), Principle 2 (0.98 score), as microliter sample volumes are added to the 3MPA-CdTe QDs probes; Principle 4 (1.0 score), due to the limited number of steps involved; Principle 6 (1.0 score) as no derivatization procedure is required, and Principle 9 (1.0 score) since the energy consumption of the method is minimal. Average performance (yellow range color) was achieved for Principle 5 (0.5 score) as the method is non automated but prone for semi automation and with miniaturized characteristics; Principle 8 (0.51 score) as the method is focused on a single analyte but enable analyzes of about 10 samples/h. Higher penalties were found for Principle 1 (score 0.3) as external sample treatment and batch analysis are used although measurements could be semi-automated; Principle 3 (0.33 score) as the method is at line; Principle 7 (sore 0.39) as small amount of toxic reagents is regenerated as waste; Principle 11 (0.29 score) as it involves small amounts of toxic reagents, and Principle 12 (0.4 score) as some operation safety issues can be raised. Finally, to Principle 10 a zero score is attributed due to the lack of bio-based reagents involved.

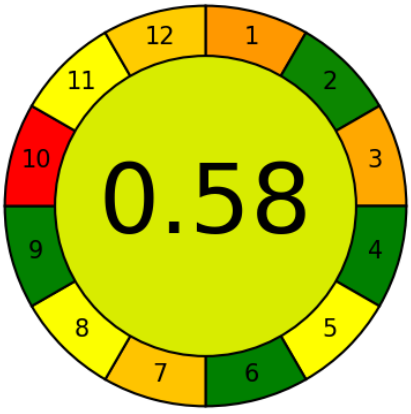

Figure 4. Pictogram from the AGREE calculator concerning the proposed method.

CONCLUSIONS

A novel analytical method was developed aiming the determination of quercetin based on the photoluminescence quenching of 3MPA-CdTe QDs. The method was applied in food supplement and manipulated formulation. Although designed as a screening method, results achieved satisfactorily agreed

with the ones obtained using HPLC. The method was found to be simple even if a prior TLC procedure to separate analyte is required prior to determination. Adaptation to an automatized high performance thin-layer chromatography (HPTLC) would improve selectivity, analytical frequency and reproductibility of results. The method is cost effective, producing low quantities of waste as it requires low amount of organic solvents, micromolar amounts of the 3MPA-CdTe QDs also requiring small quantities of samples. The simplicity of the method to quantify quercetin content may be affected by co-migration of other polyphenols (usually present at lower concentrations than QUE) when TLC is applied. However, the use of high-performance thin-layer chromatography will certainly improve selectivity in separation prior to detection, being this a valid adaptation if greater guarantee of selectivity is required.

Conflicts of interest

Authors declare no financial conflict.

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