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# Rapid detection of vegetable cooking oils adulterated with inedible used oil using fluorescence quenching method with aqueous CTAB-coated quantum dots

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ABSTRACT

Vegetable cooking oils adulterated with inedible used oils or impure oils have posed a severe food safety problem. Current detection methods cannot meet the needs for rapid detection of adulterated oils on line or in field. Therefore, the objective of this research was to develop a rapid optical sensing method based on fluorescence quenching of CdSe/ZnS quantum dots (QDs) for identification of adulterated vegetable cooking oils. High quality hydrophilic photoluminescent nanoparticles were synthesized by encapsulating hydrophobic QDs into the micellar structure of an amphiphilic surfactant cetyltrimethyl ammonium bromide (CTAB) via phase transfer method. TEM, AFM, and fluorescence spectroscopy were used to characterize the prepared CTAB-coated QDs. Oil samples were first captured into the core of the two-layer-structural micelle and then the fluorescence of CTAB-coated QDs, working as fluorescence probes, was selectively quenched by components of the adulterated oils. Heavy metal ions and free radicals were presumed to be main quenchers. After quenching for 1 min, fluorescence intensity was measured and converted to quenching percentage to determine the adulteration concentration. The results showed that in comparison with oil-soluble QDs, water-soluble CTAB-coated QDs had a greater ability to identify oil adulteration. A good quantitative relationship between quenching percentage and adulteration concentration ($y = 5.96x + 14.99$; $R^2 = 0.94$) was obtained. The sensitive, simple and low-cost sensing method did not require sample pretreatment and could detect refined used oils at 0.4% or higher concentrations in soybean oil within 2 min, showing great potentials for rapid screening of used oils and quantitative analysis of used oil adulteration in field.



## 1. Introduction

Inedible used oils refer to swill oils collected from kitchen wastes, waste cooking oils recycled from gutters, residual oils stewed from animal fats, deep frying oils, and other inedible oils. They are always polluted by patterns of chemical toxins and potential carcinogens, including heavy metal ions, peroxides, trans fatty acids, aflatoxin $B_1$, and polycyclic aromatic hydrocarbons (PAHs), depending on their sources or origins [1]. Though physical refining (filtration, washing and vacuum distillation) or chemical refining (degumming, deacidifying, decolor/bleaching and deodorization) are often used to reprocess used oils, some pollutants or impurities are difficult to be completely removed and still remain in refined used oils [1]. However, some products of vegetable cooking oils adulterated with such kind of used oils or refined used oils were found recently in the market of China [1,2]. Since long-term consumption of such contaminated vegetable oils may cause serious diseases or cancers [2], it is nationally recognized as a severe food safety problem.

Many methods have been studied for determination of used oils and identification of used oil adulteration, such as Infrared (IR) [3–5], Raman [6,7], Thin Layer Chromatography (TLC) [8], High Performance Liquid Chromatography (HPLC) [9], Gas Chromatography (GC) [10], Mass Spectrometer (MS) [11], GC/MS [12], HPLC/MS

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[13] and Nuclear Magnetic Resonance (NMR) [14]. Some of these methods target the exogenous pollutants of used oils, for example, aflatoxins and benzopyrene. However, the targeted pollutants may be removed by specific refining treatments while non-targeted pollutants may still remain in the oil, causing false-negative results in detection. Some other methods target the endogenous substances such as oxidized triglycerides, triglyceride oligopolymers, modified fatty acids, and peroxides in used oils. However, these substances may already exist in regular vegetable cooking oils, long-term stored vegetable cooking oils, and the cooking oils just used in frying foods, causing false-positive results. Other fingerprinting-based techniques combined with chemometrics were investigated for authentication of vegetable oil adulteration [15,16]. After analyzing all of the oil identification methods mentioned above, they are either poor in specificity, low in sensitivity, time consuming, or require a laboratory and a highly trained technician, which may be unsuitable for rapid detection of adulterated oils on line or in field. Therefore, it is critical to develop a cost-effective authentication method with simple sample preparation for rapid screening of contaminants in vegetable cooking oils to ensure food safety and quality.

Biosensors have a great potential for disposable or portable devices used in rapid detection [17]. They are recognized as good alternatives to sensitive laboratory-based techniques in some situations [18]. Sensors like electronic noses [19–21] and thin film based chips [22] have been reported for identification and classification of vegetable cooking oils. Nanomaterials such as gold nanoparticles [23], quantum dots [24], and carbon-based nanomaterials [25] have been investigated in biosensing strategies to enhance target recognition, amplify the signal, and improve the sensitivity. Functional nanomaterial-based biosensors have shown an increasing potential to detect chemical residues, toxins, heavy metals, and organic contaminants in food samples [26]. Among them, semiconductor quantum dots (QDs) and functional QDs have been studied for their excellent optical and electronic properties. One of QDs' applications in biosensing methods is fluorescence quenching. As already reported, in aqueous solutions, the fluorescence intensity of QDs could be effectively quenched by heavy metal ions like $Pb^{2+}$ [27], $Cu^{2+}$ [28–30], or free radicals [31,32]. Our preliminary tests indicated that several components or contaminants contained in used oils, such as heavy metal ions, free radicals, electron withdrawing groups, and conjugated carbon–carbon double bonds, could also quench the fluorescence of QDs.

Therefore, in this study, a rapid and low-cost sensing method based on fluorescence quenching of CdSe/ZnS quantum dots (QDs) was investigated for detecting refined used oils. High quality hydrophilic photoluminescent nanoparticles were synthesized by encapsulating hydrophobic QDs into the micellar structure of an amphiphilic surfactant cetyltrimethyl ammonium bromide (CTAB) via phase transfer method in an emulsion system. The prepared CTAB-coated QDs were characterized by TEM, AFM and fluorescence spectroscopy. Fluorescence quenching with CTAB-coated QDs as fluorescence probes were studied. The abilities of oil-soluble CdSe/ZnS QDs and water-soluble CTAB-coated CdSe/ZnS QDs in discriminating used oils were compared. And in order to verify this method, dozens of real used oil samples and vegetable cooking oils were analyzed.

## 2. Experimental

### 2.1. Reagents

Oil-soluble CdSe/ZnS core/shell quantum dots (QDs) with trioctylphosphine oxide (TOPO) as surface stabilizing ligands and hexane as the solvent were purchased from Jiayuan Quantum Dots Co., Ltd. (Wuhan, China). Cetyltrimethyl ammonium bromide (CTAB) was obtained from Sangon Biotech Co., Ltd. (Shanghai, China). Sodium hydroxide (NaOH) and hexane were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All the reagents used were of analytical grade. All solutions were prepared with double-distilled water produced by a Milli-Q Ultrapure water system (Millipore, Billerica, USA) with the water outlet operating at 18.2 MΩ cm.

### 2.2. Instrumentation and characterization

Transmission electron microscopy (TEM) imaging was carried out for characterization of QDs and CTAB-coated QDs using JEM-1230 (JEOL, Akishima, Japan) at 80 kV. For TEM sample preparation, a drop of the diluted QDs solution or a drop of CTAB-coated QDs solution after negative staining was added to a carbon coated grid and then dried in a fume hood. More morphology observations were also performed using a Dimension Icon atomic force microscope (AFM) system (Bruker, Santa Barbara, CA, USA) with tapping mode. The samples for AFM imaging were prepared by depositing a drop of diluted QDs solution on a freshly cleaved mica surface and drying at room temperature in air overnight before observation. The ultraviolet and visible (UV–vis) absorption spectra were obtained using Agilent 8453 UV–Visible Spectroscopy System (Agilent Technologies, Santa Clara, CA, USA). The fluorescence spectra were collected using Synergy H1 Hybrid Multi-Mode Microplate Reader with Gen5 2.0 Data Analysis Software (BioTek Instruments, Winooski, VT, USA). For the fluorometric analysis, flat bottom 96-well plates (Corning Shanghai Company, Ltd., Shanghai, China) were used.

### 2.3. Oil samples

Bottled first-grade vegetable cooking oils, including corn oil (Brand A, China; Brand F, China; Brand C, China; Brand X, China), rapeseed oil (Brand A, China), sunflower seed oil (Brand A, China), and edible blended oil (Brand A, China; Brand F, China) were purchased from local grocery stores. Three first-grade soybean oils in bulk were provided by an oil company in Shandong Province, China. Real used oils from different cities in China, including six crude used oils (without any refining), four physical refined used oils (filtered; washed with water for two times; vacuum distillated), nine bleached used oils (degummed; deacidified; decolorized/bleached) were provided by State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang, China. One refined used oil (degummed; deacidified; decolorized/bleached; deodorized) was obtained from a local market. The used oil samples were not qualified for physical and chemical characteristics, such as the aflatoxin $B_1$ and benzo (a) pyrene contents, according to the National Standard of the People's Republic of China, GB 2716-2005 *National Hygienic Standard for Edible Vegetable Oil* [2,8]. All the oil samples are listed in Table 1.

### 2.4. Synthesis and characterization of CTAB-coated CdSe/ZnS QDs

The water-soluble CTAB-coated CdSe/ZnS QDs were synthesized according to the protocols described by Fan et al. [33] with necessary modification as follows. The surfactant aqueous solution was obtained by fully dissolving CTAB (1.652 g) in deionized water (45 mL) in an ultrasonic water bath (40 °C) for 20 min, making a $10^{-3}$ dilution, and adjusting the pH to 9.5 with a 20% ($w\,w^{-1}$) solution of NaOH. Secondly, 0.2 mL of 8.0 μM CdSe/ZnS QDs hexane suspension was slowly added into 30 mL of the as-prepared CTAB aqueous solution drop by drop within 30 min under vigorous stirring (900 rpm, WIGGENS hot plate/stirrer WH220 Plus, Shinetek Instruments Co. Ltd., Beijing, China). Finally, a

**Table 1**
Results of the tests for qualitative and quantitative analysis of the real used oil samples and vegetable cooking oils.

| Sample | Origin | Quenching (%) | Level | Predicted amount of adulteration (%) |
|---|---|---|---|---|
| 1 | Crude used oil-HZ-1[a] | 74.8 ± 0.9[b] | +++ | 10.0[c] |
| 2 | Crude used oil-NC-1 | 70.5 ± 1.0 | +++ | 9.3 |
| 3 | Crude used oil-NC-2 | 87.4 ± 1.3 | ++++ | 12.2 |
| 4 | Crude used oil-JJ-1 | 63.3 ± 0.7 | ++ | 8.1 |
| 5 | Crude used oil-YC-1 | 72.1 ± 0.5 | +++ | 9.6 |
| 6 | Crude used oil-YT-1 | 86.8 ± 1.5 | ++++ | 12.1 |
| 7 | Physical refined used oil-1 | 60.8 ± 1.0 | ++ | 7.7 |
| 8 | Physical refined used oil-2 | 84.8 ± 0.8 | ++++ | 11.7 |
| 9 | Physical refined used oil-3 | 73.1 ± 0.6 | +++ | 9.8 |
| 10 | Physical refined used oil-4 | 81.7 ± 2.5 | ++++ | 11.2 |
| 11 | Bleached used oil-HZ-1 | 92.8 ± 0.7 | +++++ | 13.1 |
| 12 | Bleached used oil-NC-1 | 78.8 ± 1.6 | +++ | 10.7 |
| 13 | Bleached used oil-NC-2 | 57.5 ± 1.1 | ++ | 7.1 |
| 14 | Bleached used oil-NC-3 | 57.9 ± 1.5 | + | 7.2 |
| 15 | Bleached used oil-YC-1 | 79.4 ± 1.1 | ++ | 10.8 |
| 16 | Bleached used oil-YC-2 | 65.9 ± 0.6 | ++ | 8.5 |
| 17 | Bleached used oil-YC-3 | 53.7 ± 1.0 | + | 6.5 |
| 18 | Bleached used oil-YT-1 | 74.8 ± 1.5 | +++ | 10.0 |
| 19 | Bleached used oil-YT-2 | 63.4 ± 1.3 | ++ | 8.1 |
| 20 | Refined used oil-1 | 58.4 ± 2.2 | + | 7.3 |
| 21 | Corn oil, Brand A | 74.1 ± 0.7 | +++ | / |
| 22 | Corn oil, Brand F | 71.1 ± 0.8 | +++ | / |
| 23 | Corn oil, Brand C | 79.4 ± 1.5 | +++ | / |
| 24 | Corn oil, Brand X | 34.6 ± 2.2 | – | / |
| 25 | Edible reconciling oil, Brand A | −29.7 ± 1.4 | – | / |
| 26 | Edible reconciling oil, Brand F | −16.2 ± 5.0 | – | / |
| 27 | Rapeseed oil, Brand A | −69.9 ± 4.2 | – | / |
| 28 | Sunflower oil, Brand A | 34.0 ± 1.3 | – | / |
| 29 | Soybean oil in bulk 1 | −47.5 ± 6.9 | – | / |
| 30 | Soybean oil in bulk 2 | −29.2 ± 1.2 | – | / |
| 31 | Soybean oil in bulk 3 | −37.4 ± 3.7 | – | / |

[a] HZ, NC, JJ, YC, YT represents different cities where the oil samples were collected.
[b] Mean ± standard deviation ($n \geq 3$).
[c] "+" represents positive result, indicating the tested oil sample is used oil/impure oil. "+", "++", "+++", "++++", "+++++" represent positive one (50–60%), positive two (60–70%), positive three (70–80%), positive four (80–90%), and positive five (>90%), respectively. "–" represents negative result (<50%), indicating the tested oil sample is not a used oil-adulterated one.

homogeneous oil-in-water microemulsion was obtained. Then the mixture was heated at 50 °C under vigorous stirring (450 rpm, WIGGENS hot plate/stirrer WH220 Plus) for another 30 min to evaporate the hexane, transferring the QDs into the aqueous phase. Thus, a clear solution of water-soluble surfactant-coated QD micelles was formed.

The fluorescence spectra were recorded to characterize the QDs and CTAB-coated QD micelles. TEM images and AFM images were taken to further confirm the diameters and shapes of the prepared QDs coated with surfactant CTAB.

### 2.5. Fluorescence quenching assay procedure

A 30 μL oil sample was added into 300 μL of QDs solution (hexane as solvent) or CTAB-coated QDs solution (ultrapure water as solvent) in a 1.5-mL microcentrifuge tube. This solution was vigorously stirred with a "Vortex-Genie 2" Mixer (Scientific Industries, Inc., New York, USA) for 1 min. Then, 100 μL of the mixture was transferred into flat bottom 96-well plate. Fluorescence emission spectrums were measured under the maximum excitation wavelength with the programmable microplate reader. The temperature of the assay was set at 25 °C. Each sample was tested in duplicate. The fluorescence quenching assay procedure was repeated three times.

### 2.6. Blend preparation

The adulterated vegetable oils with concentrations (V/V) of inedible used oils at 0%, 0.4%, 2.0%, 6.0%, 10.0%, and 13.0% were prepared by blending 0, 4, 20, 60, 100, and 130 μL of typical inedible used oils with 1000, 996, 980, 940, 900, and 870 μL of soybean oils, respectively. The prepared adulterated oil samples were mixed for at least 1 min with a "Vortex-Genie 2" Mixer before use.

### 2.7. Calibration curve

The fluorescence intensities were measured for the samples with a concentration of inedible used oils at 0% (control sample in this research, pure soybean oil) and five mimetic adulterated oil samples with concentrations of inedible used oils at 0.4%, 2.0%, 6.0%, 10.0%, and 13.0% according to the procedures described in Section 2.5. Quenching percentages were calculated according to the following equation:

$$Q_x = \frac{(F_0 - F_x)}{F_0} \times 100\%$$

where $Q_x$ is quenching percentage when the adulteration concentration is $x$; $F_0$ is the fluorescence intensity of control sample; $F_x$ is the fluorescence intensity of detected oil samples with adulteration concentration $x$. Then quenching percentage $Q_x$ is plotted as a function of adulteration concentration. The calibration curve was obtained from the linear regression line.

### 2.8. Tests on real used oil samples and vegetable cooking oils

The samples of 20 used oils and 11 vegetable cooking oils (in bottle or in bulk) mentioned in Section 2.3 were tested according to the procedures described in Section 2.5. The means and standard deviations (SD) of the measured fluorescent intensities (three replicates) were calculated using Microsoft Excel 2010 (Microsoft, Redmond, WA, USA). Then the adulteration percentage of each used oil sample could be calculated using the calibration curve. To intuitively

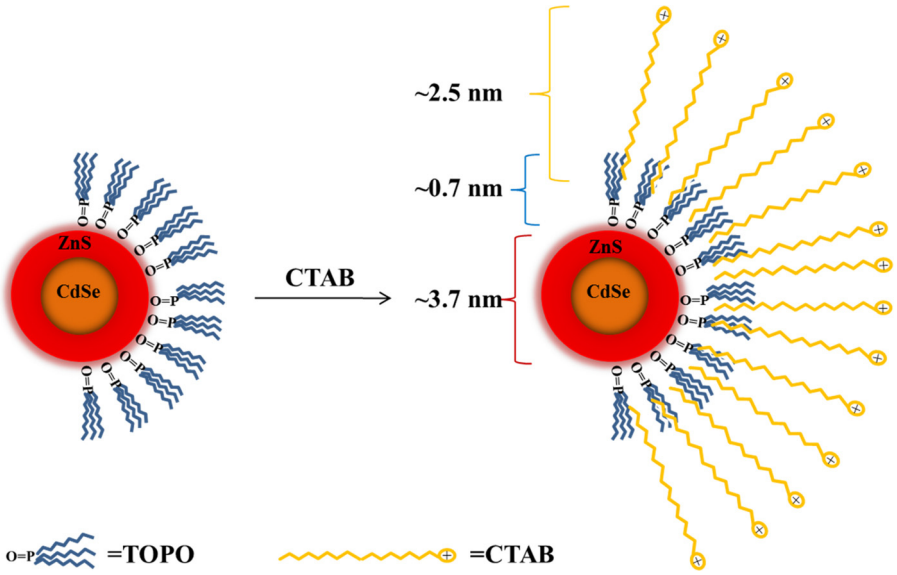



**Fig. 1.** Schematic illustration of the preparation process and the structure of water-soluble CdSe/ZnS QDs with CTAB as a stabilizing reagent.

determine whether an oil sample was adulterated with used oils, as well as the degree of adulteration, the results were classified into six levels according to the quenching percentages, namely, negative "−" (<50%), positive one "+" (50–60%), positive two "++" (60–70%), positive three "+++" (70–80%), positive four "++++" (80–90%), and positive five "+++++" (>90%), as shown in Table 1.

## 3. Results and discussion

### 3.1. Characterization of CTAB-coated CdSe/ZnS QDs

Cationic surfactant CTAB was chosen as the phase transfer agent to convert QDs from organic phase to aqueous phase [34]. The experimental process of preparing monodispersed water-soluble CTAB-coated CdSe/ZnS QDs is schematically illustrated in Fig. 1. Spherical micelles of CTAB with the cationic quaternary amine headgroup facing outward towards the aqueous medium at critical micelle concentration were prepared in alkaline solution forming an oil-in-water microemulsion [35]. When concentrated suspensions of original QDs in hexane were introduced gradually into the CTAB solution, the oil droplets were stabilized by the CTAB micelles. It should be pointed out that there might be one or more QD particles packed into one single CTAB micelle [36]. Subsequently, under heating and vigorous stirring conditions, the volatile solvent hexane was evaporated gradually and it drove the hydrophobic long alkyl chain of CTAB molecule and surface ligand TOPO molecule at the surface of every single QD to intercalate each other through van der Waals' interactions, resulting in water-soluble CTAB-coated monodispersed QDs. The cationic surfactant CTAB played an important role in making hydrophilic QDs which were originally stabilized by oil-soluble TOPO soluble in aqueous solution. In addition, sodium hydroxide not only helped create an alkaline environment in favor of the formation of CTAB micelle, but also benefited the layer of CTAB being packed more densely [37]. Since the initial capping ligand TOPO was not removed, it was not a ligand exchange process [38–40], but a phase transfer process.

The fluorescence spectra were recorded under the same conditions to characterize the QDs and CTAB-coated QD micelles. Fig. 2A displays the fluorescence intensity of the modified CTAB-coated CdSe/ZnS QDs in water phase only decreased a little (less than 10%) in comparison with the original QDs in hexane. This was due to the fact that both the fluorescence quantum efficiency and the absolute non-radiative quantum efficiency were dependent on the core size of the CdSe/ZnS QDs, so that the functionalization with amine groups would not significantly change the high quantum efficiency values [41,42]. As shown in Fig. 2A, the maximum emission wavelength of newly obtained QDs was found at 610 nm, whereas that of the original one was 605 nm. The 5 nm red shift was due to the slightly enlarged particle size of the CTAB-coated QDs after the original QD was incorporated into the secondary surfactant layer. The differences in intensity and the red shift as compared to the controls suggest the occurrence of interdigitation as mentioned before. To further confirm the diameters and shapes of the prepared single QD coated with surfactant CTAB, AFM images and TEM images were taken. Fig. 2B shows AFM images and the representative height profile of control QDs (i) and the prepared CTAB-coated QDs (ii). The height profiles confirm the QDs with a diameter of 3 ~ 4 nm and the CTAB-coated QDs with a diameter of 9 ~ 10 nm were both uniform and monodispersed. The results were in accordance with the size of CdSe/ZnS/TOPO QDs (Fig. 1) reported by Xu et al. [43]. TEM images (Fig. 2C) reveal that the QD particles with a diameter of approximately 4 nm in hexane (i) and the CTAB-coated QD particles with a diameter of approximately 10 nm in water (ii), which were coherent with those of AFM studies. It should be pointed out that several clusters were observed in the TEM image of the CTAB-coated QDs (Fig. 2C, ii), but not in the AFM image of the CTAB-coated QDs (Fig. 2B, ii). They might be clusters of CTAB-coated QDs as background interferences caused by the negative staining treatment for TEM. Fig. 2D shows that under identical experimental conditions, the fluorescence intensity of the 5-month-long stored CTAB-coated QDs (ii) under $UV_{365}$ were almost the same as the freshly prepared ones (i), indicating that the hydrophilic photoluminescent nanoparticles were stable and highly preserved. Additionally, supported by the fact of the encapsulation of one single QD into one surfactant micelle, fluorescence self-quenching caused by high concentration of QDs would not happen.

### 3.2. Fluorescence quenching of CTAB-coated CdSe/ZnS QDs

Fig. 3 shows a schematic display of a QDs-based fluorescence quenching mechanism. In this optical sensor, CTAB-coated QDs were employed as fluorescence probes in aqueous solution. The inner part of the micelle was oil phase and the outer part was water

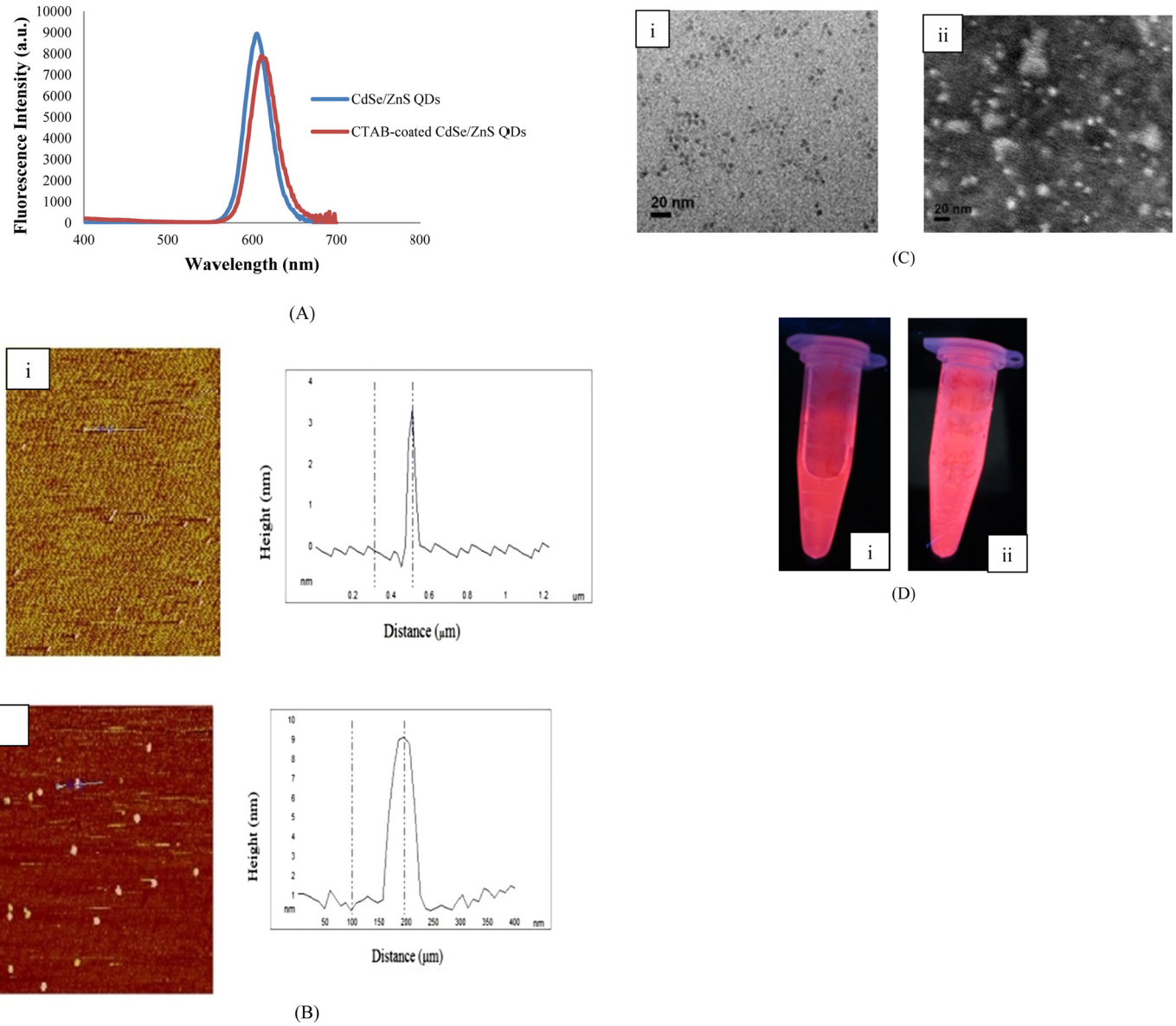



**Fig. 2.** (A) Emission fluorescence (FL) spectra of CdSe/ZnS QDs (blue line) and CTAB-coated CdSe/ZnS QDs (red line). The FL spectra were recorded under the same condition. The maximum emission wavelength of CdSe/ZnS QDs was 605 nm and that of CTAB-coated CdSe/ZnS QDs was 610 nm. (B) Tapping mode AFM images and the height profiles of the control CdSe/ZnS QDs (3 ~ 4 nm thick) (i) and CTAB-coated CdSe/ZnS QDs (9 ~ 10 nm thick). (ii) Both were uniform and monodispersed. (C) TEM images of CdSe/ZnS QDs with a diameter of approximately 4 nm in hexane (i) and CTAB-coated CdSe/ZnS QDs with a diameter of approximately 10 nm in water (ii). (D) The fluorescence intensity under $UV_{365}$ of freshly prepared CTAB-coated QDs (i) and that of 5-month-long stored ones (ii). Pictures were taken under the same condition. They present almost the same intensity, indicating CTAB-coated CdSe/ZnS QDs were stable and highly preserved.(For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

phase. When oil samples were added, they were captured into the core of the micelles after agitation and incubation on a Fisher Vortex Genie 2 for 1 min. As a result, the QDs were quenched by fluorescence quenchers and it could be demonstrated by the reduced fluorescence intensity of fluorescence probes at the wavelength of 610 nm (emission) when excited at the wavelength of 305 nm. Visually, as elucidated in the inserted picture in Fig. 3A and B, the QDs solution went from red to blue under $UV_{365}$, indicating the fluorescence quenching occurred in the fluorescence sensor. It should be mentioned that the blue color was just the background color of the sample (the color of CTAB solution) when observing under UV365 in a dark box, but not the color that emitted from the QDs itself.

Fig. 4A shows six emission spectrum of CTAB-coated QDs solution quenched by five adulterated oil samples with different concentrations of refined used oils ranging from 0% to 13%. Results revealed that the quenching increased gradually with the concentration of adulteration ($y = 5.96x + 14.99$; $R^2 = 0.94$). Fig. 4B indicates that there was a good linear relationship between adulteration concentration and quenching percentage with 0.94 of the coefficient of determination. The lower detection limit was determined by multiplying the standard deviation of the control quenching by three and was found to be 0.4%. Thus, this method could detect 0.4% or higher concentration of inedible used oils in soybean oil. This fluorescence quenching method achieved lower detection limit than the two reported rapid detection methods, the ultraviolet-visible absorption spectroscopy method [44] and fluorescence spectroscopy method [45]. Both of these methods could distinguish 10% or more adulteration of used oils in corn oils (the same fluorescence intensity). It proved that the proposed CTAB-coated QDs based optical sensing method was more sensitive and

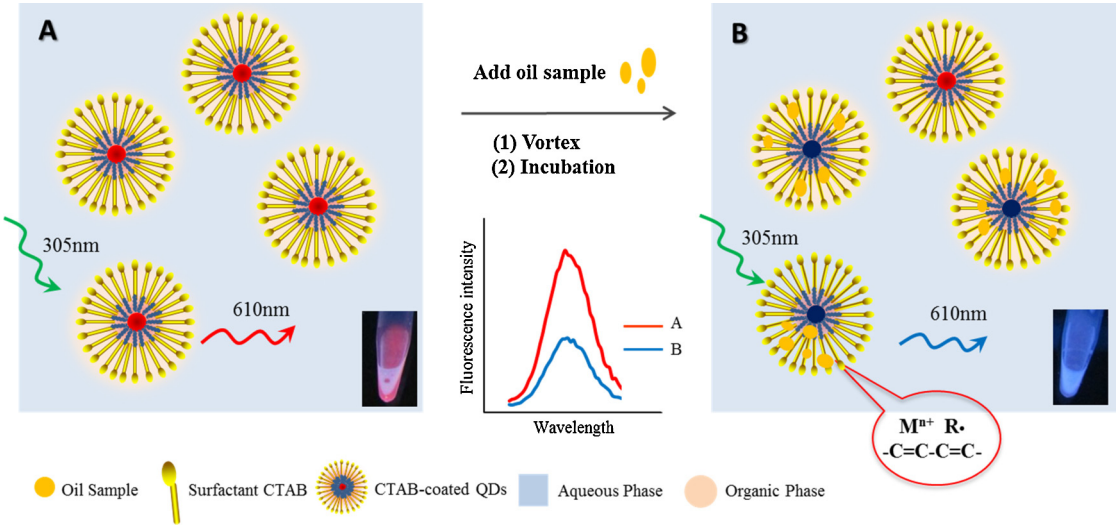
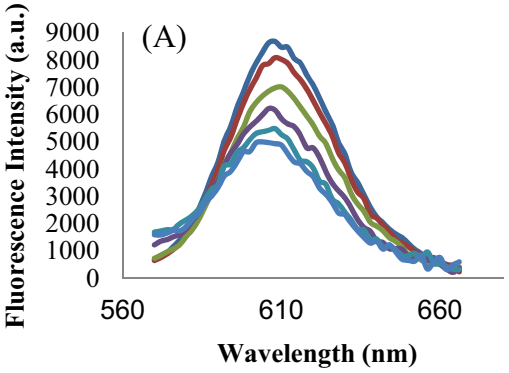



**Fig. 3.** A schematic diagram of QDs-based fluorescence quenching mechanism ($M^{n+}$, R•, —C=C—C=C— represent heavy metal ions, free radicals, and conjugated carbon–carbon double bonds, respectively).

effective. Moreover, the detection of adulterated vegetable cooking oils did not require a sample pretreatment, and only 10 μL of sample volume was required. The total detection process could be finished within 2 min. The fluorescence quenching method could not only qualitatively determine whether vegetable cooking oil was adulterated, but also quantitatively analyze the adulteration degree.



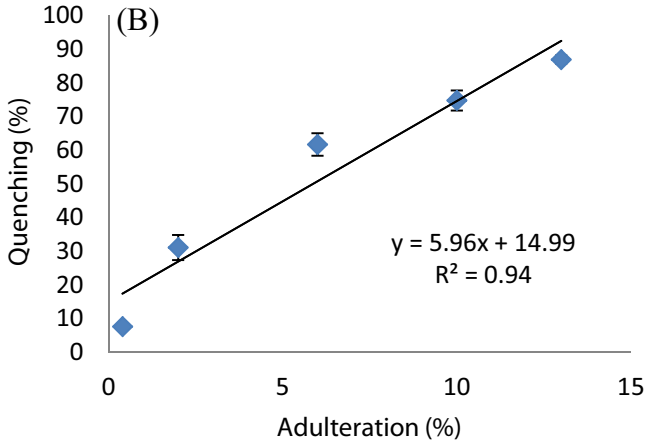




**Fig. 4.** (A) Six emission spectra of CTAB-coated QDs solution quenched by five adulterated oil samples with concentrations of 0%, 0.4%, 2.0%, 6.0%, 10.0% and 13.0%. (B) The quantitative relationship between adulteration and quenching. The means and error bars (standard deviation) were calculated based on three replicates.

It should be pointed out that inedible used oils with different origins had different compositions and different refining treatment would also result in different used oils, there's almost no standard used oil sample right now [1,2,6,8]. Actually, for the identification of adulteration of used oils, lack of standard samples is one of the biggest challenges that most researchers have been facing with and one of the main reasons why it's so difficult to accurately determine adulterated oils. The calibration curve was obtained only based on the samples collected and not validated with other different oils, which is the limitation of this study.

### 3.3. Comparison of oil-soluble QDs and water-soluble CTAB-coated QDs

The results of the same fluorescence quenching tests with adulterated oil samples using oil-soluble QDs in hexane solution compared with that of CTAB-coated QDs in water solution are presented in Fig. 5. When quenched by the adulterated oils, the quenching percentage of both showed increasing trends with the adulteration concentration. For CTAB-coated QDs, quenching was 10.8% when adulteration concentration was 0% and quenching

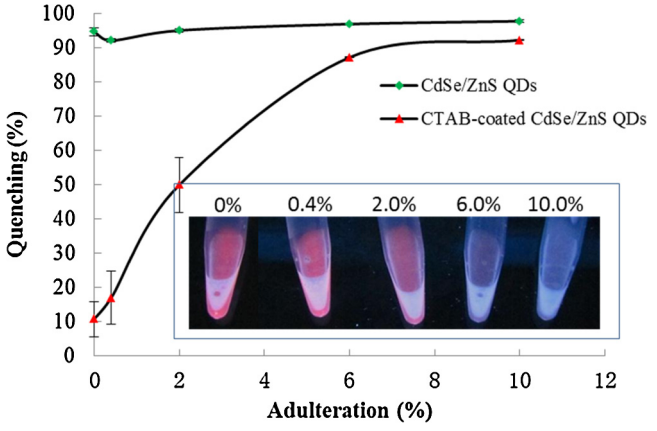



**Fig. 5.** The relationship between adulteration and quenching using CdSe/ZnS QDs and CTAB-coated CdSe/ZnS QDs as quenching probes, respectively. The picture shows quenching phenomenon observed in QDs solutions with different percentages of adulteration. The means and error bars (standard deviation) were calculated based on three replicates.

percentage moved sharply to 92.2% when adulteration concentration increased to 10%, whereas for CdSe/ZnS QDs, quenching percentage only increased a little (from 94.6% to 97.7%). It meant that original QDs were almost completely quenched by components in oil samples, no matter how much used oils adulterated. While the prepared CTAB-coated QDs were partially quenched by oil samples and the quenching degree was determined by the adulteration concentration. Therefore, water-soluble CTAB-coated CdSe/ZnS QDs possessed a stronger ability to identify oil adulteration than oil-soluble CdSe/ZnS QDs.

Though the shape or size might be slightly changed, the modified QDs could hold original optical properties after the surface functionalization by amphiphilic surfactant as a second protection [42]. The interdigitated bilayer structure of CTAB-TOPO-coated CdSe/ZnS QD, whose first layer was original surface ligands TOPO and the second layer was CTAB, made it a more sensitive and selective fluorescence probe than CdSe/ZnS QD to the quenchers [46]. Similar organic coated bilayer structures of water-soluble CdSe/ZnS QDs were also investigated by others as fluorescence quenching probes for selective detection and quantification of analytes like chloride anion [47], hypochlorite [48], heavy metal ions [27–30,49], nitroaromatic [50] and even chiral mixtures of enantiomers [51,52].

Through the comparison, it is speculated that the differences in sensitivity of the two types of sensing probes were first due to the selective quenching of CTAB-coated QDs by hydrophobic quenchers contained in oil samples. They could easily dissolve into the oil phase in the inner part of the micelle structure. The hydrophilic ones, however, were dissolved in the bulk solution. Secondly, the high sensitivity was also attributed to the selective quenching of CTAB-coated QDs by small molecule quenchers who possessed very tiny spatial structure. Their smaller size allowed easier diffusion through the two-layer micelle structure and made it closer to the surface of CdSe/ZnS core/shell structure. The larger ones, however, were blocked in the outer layer of CTAB, serving as a protecting layer. In addition, though the cationic quaternary amine of CTAB was facing outward, the positively charged surface of CTAB-coated QDs was neutralized electrically by the free hydroxyl groups in alkaline solution, which made it unattractive to those negatively charged quenchers or repulsive to those positive ones such as heavy metal ions [40].

Numerous chemical species in oil samples could act as fluorescence quenchers to QDs. As reported by Lepri et al. [53], trace metal elements, such as Co, Mn, Ni, Cd, Hg, Pb, can be naturally present in vegetable oils or become incorporated due to extraction and refining processes, as well as due to environmental contamination. Used oils that had contacted with metal ware when they were heated for frying food and held for storage and transportation, were severely contaminated by heavy metal ions, such as $Pb^{2+}$, $Cr^{6+}$, $Cu^{2+}$, $Mn^{2+}$, $Zn^{2+}$, $Ni^{2+}$ [54]. The $Pb^{2+}$ [27] and $Cu^{2+}$ [28–30] were demonstrated to decrease the fluorescence intensity of functional ligands capped CdSe/ZnS QDs. One mechanism of the fluorescence quenching of surface modified inorganic CdSe/ZnS QDs was the cation exchange of Zn with the quenching heavy metals. This was driven by the difference in relative electronegativities of metallic elements, bond energies of metal-sulfur, formation energies of the sulfide compounds, as well as the difference in solubility of the sulfide compounds [55]. Another mechanism of the quenching of modified QDs by heavy metal ions may be due to the coordination of heavy metal ions with the capping material at the surface of QDs, resulting in the electron transfer from excited functionalized QDs to heavy metal ions [56].

The oxidization and deterioration occurring in oils during high-temperature usage or long-term storage would release some harmful free radicals. It was reported that free radicals could quench the fluorescence of QD nanoparticles [31], and the quenching of QD luminescence by radicals was non-linear [57,58]. For the quenching of QD luminescence by organic radicals, a mechanism of the ligand exchange of TOPO with radicals was speculated by Heafey et al. [32]. Recently a new mechanism was proposed requiring close proximity for electron transfer or spin exchange between the radical (electron acceptor) and the excited electron in the conduction band of QD (electron donor) [59].

Nevertheless, taking all these into consideration, heavy metal ions and free radicals with small molecular structures could be speculated to be the most effective and typical fluorescence quenching reagents in this CTAB-coated QDs-based fluorescence sensing method with high sensitivity and selectivity for steric hindrance, solubility, and electric properties. Some other chemicals in used oils might also snatch electrons in the conduction band, fill the holes in the valence band of excited QDs, or damage the perfect surface of QDs, resulting in blocked recombination of electron–hole pairs and a decrease in radioactive emission or fluorescence intensity of QDs. That is to say there may be several quenching pathways in this fluorescence sensor using CTAB-coated CdSe/ZnS QDs as probes. As acknowledged, there are thousands of chemical or biological molecules in used oil, making it a complex matrix. Intricate chemical reactions between them under heat and light, or in the presence of oxygen make it even more complicated. However, few studies have revealed what are the exact effective components at molecular level in oils and how they cause fluorescence quenching of QDs. To answer this question, further fundamental researches are still needed.

### 3.4. Detection of real used oil samples

In order to validate the sensor, dozens of inedible used oils were tested. The results of the analysis of real used oil samples are summarized in Table 1. Based on the quenching results, the 20 used oil samples were all identified as positive, namely 100% of the accuracy of positive identification. Among the 11 cooking oils, three of them were false positive, resulting in 72% of the accuracy of negative identification. And according to the quenching degree, the positive results could be further qualitatively classified into five levels, and the amount of adulteration could be quantitatively predicted.

Used oil samples came from different places and were treated by different refining processes. Therefore, they contained different amounts of targeted quenching components, resulting in different quenching abilities. Crude used oils were not treated by any refining process and contained lots of quenchers, showing higher quenching ability. Since physical refining could not remove effective quenchers including heavy metal ions away from used oils, they also showed higher quenching result. For bleached used oils, the refining processes of degumming, deacidifying, and decoloring could get rid of lots of impurities, leading to reduced quenchers in bleached oil samples, showing relatively low quenching value. For the well-refined used oil, since it was produced from bleached used oils by deodorizing, making it relatively more pure, the quenching value was the lowest among all the used oils. Based on these results, it was observed that the refining processes could not completely clear all of the quenchers away from every oil sample. For the 11 vegetable cooking oils, three out of four corn oils were identified as positive, while the corn oil of Xiwang Brand, together with the rest control oil samples, was negative. Further research is needed to explain why only corn oil caused false-positive results in the tests using this proposed method. More used oil samples should be used in the tests for further validation of the method.

## 4. Conclusions

A sensitive optical sensing method was developed in this study for rapid identification of vegetable cooking oils adulterated with

used oils using a fluorescence quenching method without any sample pretreatment. High quality hydrophilic CTAB-coated CdSe/ZnS QDs, working as fluorescence probes, were synthesized through the phase transfer method by encapsulating hydrophobic QDs into the micellar structure of amphiphilic surfactant CTAB. Through comparison, water-soluble CTAB-coated QDs were found to possess a stronger ability to identify oil adulteration than oil-soluble QDs. The sensitive and selective detection of the adulterated edible oils was achieved through fluorescence quenching method based on CTAB-coated QDs in the aqueous solution. A concentration of 0.4% or higher of used oils in soybean oils could be quantitatively detected using this rapid, simple and low-cost detection method within 2 min. The simple and sensitive QDs-based optical method has potential for rapid and qualitative screening of used oils and quantitative analysis of used oil adulteration in field. Future research is needed for the evaluation of this method with a broader range of different vegetable cooking oil samples and for the development of an instrument for this optical sensor.

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## References

[1] W. Cao, X. Sun, F. Chen, B. Xue, W. Chen, Q. Jin, X. Wang, Chin. Oil. Fat. 37 (2012) 1–5 (in Chinese).
[2] J. Huang, H. Xiong, L. Li, C. Luo, Chin. Oil. Fat. 33 (2008) 70–74 (in Chinese).
[3] C.M. McGoverin, D.J. September, P. Geladia, M. Manley, J. Near Infrared Spectrosc. 20 (2012) 521–528.
[4] A. Rohman, Y.B. Che-Man, Food Chem. 129 (2011) 583–588.
[5] A.S. Luna, A.P. Da-Silva, J.S. Pinho, J. Ferre, R. Boque, Spectrochim. Acta A 100 (2013) 115–119.
[6] W. Feng, L. Fu, X. Sun, X. Tian, C. Wang, H. Xiong, Y. Li, L. Chen, Modern Sci. Instrum. 3 (2012) 57–59 (in Chinese).
[7] M. Zou, X. Zhang, X. Qi, H. Ma, Y. Dong, C. Liu, X. Guo, H. Wang, Food Chem. 57 (2009) 6001–6006.
[8] J. Huang, H. Xiong, X. Xiong, Z. Chen, Food Sci. 29 (2008) 568–571 (in Chinese).
[9] M. Povolo, G. Contarini, Rivista Italiana delle Sostanze Grasse 89 (2012) 184–192.
[10] C. Ruiz-Samblas, F. Marini, L. Cuadros-Rodriguez, A. Gonzalez-Casado, J. Chromatogr. B 910 (2012) 71–77.
[11] L. Vaclavik, B. Belkova, Z. Reblova, K. Riddellova, J. Hajslova, Food Chem. 138 (2013) 2312–2320.
[12] C. Ruiz-Samblas, L. Cuadros-Rodriguez, A. Gonzalez-Casado, F.D. Garcia, P. de la Mata-Espinosa, J.M. Bosque-Sendra, Anal. Bioanal. Chem. 399 (2011) 2093–2103.
[13] V. Di-Stefano, G. Avellone, D. Bongiorno, V. Cunsolo, V. Muccilli, S. Sforza, A. Dossena, L. Drahos, K. Vekey, J. Chromatogr. A 1259 (2012) 74–85.
[14] M.F. Marcone, S. Wang, W. Albabish, S. Nie, D. Somnarain, A. Hill, Food Res. Int. 51 (2013) 729–747.
[15] J. Zhang, X. Zhang, L. Dediu, C. Victor, Food Control. 22 (2011) 1126–1135.
[16] D.I. Ellis, V.L. Brewster, W.B. Dunn, J.W. Allwood, A.P. Golovanov, R. Goodacre, Chem. Soc. Rev. 41 (2012) 5706–5727.
[17] A. Turner, Trends Biotechnol. 31 (2013) 119–120.
[18] T.F. McGrath, K. Andersson, K. Campbell, T.L. Fodey, C.T. Elliott, Biosens. Bioelectron. 41 (2013) 96–102.
[19] S. Mildner-Szkudlarz, H.H. JeleŃ., J. Food Qual. 33 (2010) 21–41.
[20] A. Berna, Sensors (Basel) 10 (2010) 3882–3910.
[21] A.M. Marina, Y.B. Che Man, I. Amin, J. Am. Oil Chem. Soc. 87 (2009) 263–270.
[22] S. Bai, S. Li, T. Yao, Y. Hu, F. Bao, J. Zhang, Y. Zhang, S. Zhu, Y. He, Food Control. 22 (2011) 1624–1628.
[23] M. Zougagh, R. Salghi, S. Dhair, A. Rios, Anal. Bioanal. Chem. 399 (2011) 2395–2405.
[24] V. Biju, T. Itoh, A. Anas, A. Sujith, M. Ishikawa, Anal. Bioanal. Chem. 391 (2008) 2469–2495.
[25] L. Wang, W. Ma, L. Xu, W. Chen, Y. Zhu, C. Xu, N.A. Kotov, Mater. Sci. Eng. R 70 (2010) 265–274.
[26] X. Zhang, Q. Guo, D. Cui, Sensors (Basel) 9 (2009) 1033–1053.
[27] W. Luan, H. Yang, Z. Wan, B. Yuan, X. Yu, S.-t. Tu, J. Nanoparticle Res. 14 (2012) 1–8.
[28] T. Sung, Y. Lo, Sens. Actuators B – Chem. 165 (2012) 119–125.
[29] M. Koneswaran, R. Narayanaswamy, Sens. Actuators B – Chem. 139 (2009) 104–109.
[30] Y. Zhang, H. Zhang, X. Guo, H. Wang, Microchem. J. 89 (2008) 142–147.
[31] K. Sill, T. Emrick, Chem. Mater. 16 (2004) 1240–1243.
[32] E. Heafey, M. Laferriere, J.C. Scaiano, Photochem. Photobiol. Sci. 6 (2007) 580–584.
[33] H. Fan, E.W. Leve, C. Scullin, J. Gabaldon, D. Tallant, S. Bunge, T. Boyle, M.C. Wilson, C.J. Brinker, Nano Lett. 5 (2005) 645–648.
[34] N.T. Thanh, L.A. Green, Nano Today 5 (2010) 213–230.
[35] B. Nikoobakht, M. El-Sayed, Langmuir 17 (2001) 6368–6374.
[36] H. Fan, K. Yang, D.M. Boye, T. Sigmon, K.J. Malloy, H. Xu, G.P. Lopez, C.J. Brinker, Science 304 (2004) 567–571.
[37] H. Bagaria, G. Kini, M. Wong, J. Phys. Chem. C 114 (2010) 19901–19907.
[38] M. Wang, M. Zhang, J. Qian, F. Zhao, L. Shen, G.D. Scholes, M.A. Winnik, Langmuir 25 (2009) 11732–11740.
[39] D. Dorokhin, N. Tomczak, M. Han, D. Reinhoudt, A. Velders, G. Vancso, ACS Nano 3 (2009) 661–667.
[40] C. Chen, S. Liu, R. Cui, B. Huang, Z. Tian, P. Jiang, D. Pang, Z. Zhang, J. Phys. Chem. C 112 (2008) 18904–18910.
[41] V. Pilla, E. Munin, J. Nanoparticle Res. 14 (2012) 1147–1154.
[42] M. Deng, N. Tu, F. Bai, L. Wang, Chem. Mater. 24 (2012) 2592–2597.
[43] J. Xu, X. Ji, K.M. Gattás-Asfura, C. Wang, R.M. Leblanc, Colloids Surf. A 284–285 (2006) 35–42.
[44] Y. Wang, P. Yin, F. Liang, L. Zhao, W. Liu, Chin. J. Anal. Lab. 25 (2006) 92–94 (in Chinese).
[45] W. Liu, P. Yin, L. Zhao, Chin. Oil. Fat. 30 (2005) 24–26 (in Chinese).
[46] J. Weaver, R. Zakeri, S. Aouadi, P.J. Kohli, Mater. Chem. 19 (2009) 3198–3206.
[47] J. Rubio, M.A. Izquierdo, M.I. Burguete, F. Galindo, S.V. Luis, Nanoscale 3 (2011) 3613–3615.
[48] Y. Yan, S. Wang, Z. Liu, H. Wang, D. Huang, Anal. Chem. 82 (2010) 9775–9781.
[49] M. Koneswaran, R. Narayanaswamy, Sens. Actuators B – Chem. 139 (2009) 91–96.
[50] R. Freeman, T. Finder, L. Bahshi, R. Gill, I. Willner, Adv. Mater. 24 (2012) 6416–6421.
[51] C. Carrillo-Carrion, S. Cardenas, B. Simonet, M. Valcarcel, Anal. Chem. 81 (2009) 4730–4733.
[52] R. Freeman, T. Finder, L. Bahshi, I. Willner, Nano Lett. 9 (2009) 2073–2076.
[53] F.G. Lepri, E.S. Chaves, M.A. Vieira, A.S. Ribeiro, A.J. Curtius, L.C. DeOliveira, R.C. DeCampos, Appl. Spectrosc. Rev. 46 (2011) 175–206.
[54] Q. Zhang, Q. Chen, Y. Liu, Z. Meng, Cereal. Oil. 9 (2010) 39–40.
[55] Y. Zhu, D. Fan, W. Shen, Langmuir 24 (2008) 11131–11136.
[56] J. Wang, X. Zhou, H. Ma, G. Tao, Spectrochim. Acta A 81 (2011) 178–183.
[57] V. Maurel, M. Laferriere, P. Billone, R. Godin, J.C. Scaiano, J. Phys. Chem. B 110 (2006) 16353–16358.
[58] M. Laferriere, R.E. Galian, V. Maurel, J.C. Scaiano, Chem. Commun. 3 (2005) 257–259.
[59] C. Tansakul, E. Lilie, E.D. Walter, F. Rivera, A. Wolcott, J.Z. Zhang, G.L. Millhauser, R. Braslau, J. Phys. Chem. C 114 (2010) 7793–7805.

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